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THE EUROPEAN RED MITE, *PARATETRANYCHUS PILOSUS* C. & F. IN NOVA SCOTIA[1]

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Abstract

Terminology, food plants, dispersion and economic importance of the European red mite, *Paratetranychus pilosus* C. & F., which affects deciduous fruit trees in America, are discussed. The mite, first observed in Nova Scotia in 1913, became of economic importance in 1925. The life history, obtained from an insectary and field study extending over a period of three years from 1930 to 1932, is recorded in detail. There are two complete generations and three partial generations in Nova Scotia. Fluctuations in temperature markedly affect mite development. Natural control of the winter eggs is important.

The European red mite, *Paratetranychus pilosus* C. & F., is a European fruit pest which was described in 1878 from Italy. It was first reported in North America by Ewing (6) in 1911, from the State of Oregon, and by Caesar (3) in 1912, from the province of Ontario. In view of the fact that it was present in such widely separated regions, it must have been introduced from Europe considerably earlier than 1911. During the following few years many new infested localities were found and at the present time the species is a pest of more or less importance in all, or at least most, of the deciduous fruit growing regions of North America.

In Nova Scotia, recollection of the presence of this mite by some entomologists can be cited as early as 1913, although at that time the species was not definitely known. In 1922, a number of orchards near the centre of the Annapolis valley were observed to be moderately infested. It was not, however, until 1925 that some special treatment to control this pest became necessary, and since then it has become increasingly imperative. At the present time it appears that the European red mite must be classed in the Annapolis valley as a permanent orchard pest which will doubtless fluctuate in numbers from year to year. Recognition of this led to a biological study of it being undertaken in Nova Scotia, during 1930, 1931, and 1932.

Terminology

It is evident from the available literature that this particular mite was described in 1878 by Canestrini and Fanzago (4) from Italy and named *Tetranychus pilosus*. In 1910, Zacher (15) erected the genus *Paratetranychus* in which he placed *pilosus*. Banks (2), in 1917, attempted to replace the genus of Zacher with *Oligonychus* of Berlese. This replacement was disputed, and an excerpt by McGregor and Newcomer (10) from Berlese, sets forth certain anatomical characters in disproof. In England, however, the species is known as *Oligonychus ulmi* and evidently workers there consider the genus of Berlese as being more applicable. Massie (8) mentions *Oligonychus ulmi* in a recent work, stating that it is known in the United States as *Paratetranychus pilosus*.

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In America, the species was first confused with the better known *Tetranychus mytilaspidis* which attacks citrus fruits. As a result, for a number of years after its appearance in 1911, it was reported as *T. mytilaspidis* by many workers, (5, 6, 12) from different parts of the United States as well as from Canada (3). McGregor (9, 10) has evidently proved conclusively that the form attacking citrus trees and that infesting deciduous fruit trees are two distinct though closely allied species. The controversy appears to have ended, at least for the time being, and the former is known as *Paratetranychus citri* McG., and the latter *Paratetranychus pilosus* C. & F.

There are also a number of common names by which this mite is known. It is only natural, considering its introduction from Europe, that it is known in America chiefly as the European red mite. It has, however, been referred to in this country as the imported red spider, and Ross (13) of Ontario mentions the plum spider mite. In England, Massie (8) refers to this mite as the fruit tree red mite. It was called the fruit tree spinning mite by Trägårdh of Sweden, while in Italy it is known as the rose mite.

Food Plants

The European red mite infests all, or at least most, of the deciduous fruits. In Ontario, Ross (13) reports that the European plum is the most important host, with the apple next. Garman (7), from Connecticut, states: "Most damage is done to apple and plum." Newcomer and Yothers (11) list plum, quince, apple, and pear as the favorite food plants. In England, Massie (8) refers to this species as probably equally important on apple and plum. In Nova Scotia, apple is the outstanding host of this mite. The writer has observed a few adult mites as well as winter eggs on plum and pear, but the numbers were few and the trees always in proximity to infested apple orchards. The fact that the plum is not seriously attacked in Nova Scotia may be explained by the comparatively small number of orchards of this fruit, or it may be that the varieties grown are those least susceptible to attack.

Host plants other than the above which have been infested include peach, walnut, cherry, almond, grape, raspberry, hawthorn, mountain ash, elm, cedar, rose and chestnut. In no instance, however, has the writer been able to find records indicating that any of these plants have been seriously infested.

Economic Importance

In the Annapolis valley, the European red mite is to be found in all districts. It is chiefly in the central and more easterly sections, however, that the worst outbreaks have occurred.

Both the adult mites and immature forms feed by withdrawing the plant juices and chlorophyll from the foliage. The leaves lose their dark green color, become pale and assume a bronze color with an unhealthy appearance. When a considerable area is infested such orchards can be detected for some distance as a result of the pale color of the foliage.

It has been stated by Garman (7) that 12 to 33 mites per leaf cause browning of apple foliage and that 55 to 133 per leaf will cause severe browning. In Nova Scotia, from 125 to 140 female adults and 90 to 226 eggs and immature forms were counted per leaf on a considerable portion of the foliage where leaf injury was severe.

As a rule the mite population is not sufficient to cause noticeable foliage injury until nearly midsummer. At this advanced date the regular spraying for other pests has been completed and it is difficult to apply effective sprays for mites so late in the season. In consequence the pest is allowed, as a rule, to proceed unchecked as far as artificial control is concerned, until the following dormant season. It is difficult to estimate the total loss as a result of severe European red mite infestations, as much depends upon the original vigor of the trees, and the treatment following the outbreaks. In orchards where the foliage has been badly depleted of chlorophyll, the fruit is late in maturing, green and small. In the autumn, green, undersized fruit is particularly noticeable on heavily set trees, which otherwise would have produced a normal crop. The foliage of infested orchards drops prematurely, resulting in depleted vigor the following year. In such orchards there is a light bloom or frequently an entire lack of blossoms.

Dispersal

In the writer's opinion there is no large scale spread of the European red mite for any distance. Once a start is made, the increase takes place, under favorable conditions, largely within the orchard itself. There are, however, ample opportunities for spread over short distances in individual orchards or to adjoining ones. Traps consisting of glass slides 3 × 5 in., smeared with Nujol oil, were exposed on stakes in such a manner that they were always facing the wind. These were placed on the east side of infested orchards so as to receive the drift of the prevailing winds. The slides were set on August 1 and left exposed for the remainder of the season. At Tupperville, N.S., three such slides were exposed 25 ft. from infested trees. Only one adult female was caught. At Lakeville, N.S., the same number of slides were exposed and placed 100 ft. from the trees and no mites were captured. Considering the small size of the exposed surface the capture of even one mite is suggestive of a wind drift of mites. In the entire investigation, however, there has been no suggestion of a wind drift of mites on a large scale.

It seems that possibly more mites are transported by light or moderate winds than by gales. A severe windstorm was experienced in the Annapolis valley on August 6 and 7, 1930, and it was observed during the storm that the mites clung very tenaciously to the foliage. On August 8, the numbers of adult female mites were counted both on the windward and the leeward sides of the trees. The average numbers of mites were 297 per 100 leaves on the windward side and 273 on the leeward side. Had there been a wind drift of any importance a concentration of mites on the leeward side would probably have followed. Both at Annapolis Royal and at Berwick the mites appeared as numerous on the foliage after the storm as before.

After a storm in September 1930, bits of webbing which had been floating in the air were found caught by apple limbs. The webbing had been spun about the massed winter eggs on the limbs at the time of egg deposition. Owing to the storm some of this webbing had been dislodged, and adhering to it were winter eggs in appreciable numbers. It would appear that this may be an important source of distribution, or at least sufficient to make a start for new infestations at some distance.

In 1930, a fruit grower at South Berwick, N.S., stated that when picking Duchess apples he noticed adult female mites in considerable numbers on a white duster suit he was wearing. If mites are brushed off the trees in this manner, it is obvious that all operations in the orchard, such as spraying, cultivating, gathering fruit, etc., are responsible for the spread about the farm or to other points where operators may be called.

The most fruitful source of spread for long distances is of course the movement of nursery stock during the dormant season. In this way the winter eggs are shipped from one country to another.

Effects of Weather on European Red Mite

All stages of the mite are susceptible to modifications by variations in weather conditions. A few warm days in May bring on hatching of the winter eggs, but a sudden lowering of temperature, or a wet cold period after hatching has begun, almost entirely suspends emergence. Further, the young mites remain in a semi-dormant condition with apparently no development throughout a cold wet period. Often they were completely submerged in drops of water on the leaves for several hours in complete inactivity. This caused no apparent ill effects, as rising temperatures with drying of the foliage soon brought renewed activity. The life of the adult is likewise prolonged and fewer eggs are deposited by the females of the first and fifth generations than are deposited by midsummer generations when higher temperature prevails. Even a cool, dull period in the warmer and more settled portion of the summer will cause at least some slight prolongation of the various stages.

It is quite evident, therefore, that there is a close correlation between the prevailing average mean temperature and the development of the immature forms. This is shown in Table I, where the average mean temperature for the period of immature development has been computed for each generation, beginning with the first mite to hatch and extending until the last deutonymph moulted and emerged as an adult. In Table II is shown the relation between the average mean temperature and the incubation period.

TABLE I

AVERAGE LENGTH OF IMMATURE PERIOD OF EUROPEAN RED MITE IN RELATION TO PREVAILING AVERAGE MEAN TEMPERATURE, ANNAPOLIS ROYAL, N.S.

Year	Average length immature period. First generation. Days	Average mean temperature. First generation. °F.	Average length immature period. Second generation. Days	Average mean temperature. Second generation. °F.	Average length immature period. Third generation. Days	Average mean temperature. Third generation. °F.	Average length immature period. Fourth generation. Days	Average mean temperature. Fourth generation. °F.	Average length immature period. Fifth generation. Days	Average mean temperature. Fifth generation. °F.
1930	(59) 17.62	53.4	(61) 9.20	66.9	(67) 11.1	66.3	(80) 11.49	63.7	(32) 15.07	59.8
1931	(42) 13.2	58.2	(60) 11.75	63.7	(54) 10.1	68.1	(57) 11.96	64.4	(49) 16.9	57.6

Figures in brackets represent the number of individuals under observation.

TABLE II

AVERAGE LENGTH OF THE INCUBATION PERIOD OF THE EUROPEAN RED MITE IN RELATION TO PREVAILING AVERAGE MEAN TEMPERATURE, ANNAPOLIS ROYAL, N.S.

Year	Average length incubation. Second generation. Days	Average mean temperature. Second generation. °F.	Average length incubation. Third generation. Days	Average mean temperature. Third generation. °F.	Average length incubation. Fourth generation. Days	Average mean temperature. Fourth generation. °F.	Average length incubation. Fifth generation. Days	Average mean temperature. Fifth generation °F.
1930	9.21	67.0	9.53	67.1	9.81	65 6	11.49	63.8
1931	13.1	60.4	8.40	67.6	9.47	66.2	13.03	60.1

Life History

The life history of the European red mite includes the following stages: egg, larva, nymph and adult. Upon the hatching of the egg there emerges a six-legged mite, the larva. After the first moult the eight-legged protonymph appears. This is followed by the deutonymph after the second moult, and upon the moulting of the deutonymph the adult emerges. During the first half of the growing season, the so-called summer eggs, which hatch during the current season, are deposited on the leaves. Later in the season, beginning about midsummer, winter eggs are deposited on the limbs. This is the stage in which the winter is passed.

In Nova Scotia, there are five generations of the European red mite, two complete and three partial ones. The females of the first and second generations deposit summer eggs only. The third deposits a small percentage of winter eggs, and the fourth deposits summer and winter eggs in the approximate ratio 60 : 40. The fifth generation deposits winter eggs only.

Method of Study

The mites were reared individually in rearing cells, according to the methods described and used by Newcomer and Yothers (11). The cells were made of white felt ¼ in. thick, cut into squares 1½ in. with a hole ½ in. in diameter punched in the centre. Squares of thin celluloid were also cut to the same size and punched in a similar manner. One of these was glued to each square of felt, a small piece of paper bearing a number for records being inserted first. A similar square of celluloid, but unpunched, was also used with each cell. These cells were attached to individual leaves on a small apple tree growing near the laboratory. The cell was placed on the under surface of the leaf, with the felt next to the leaf. The unpunched square of celluloid was then put on the upper surface and the whole fastened to the leaf with paper clips. In this way a small circle of leaf was left exposed in the felt cell. To avoid escape of the mites a thin smearing of "tanglefoot" was put on the felt around the cell. To prevent the cells being blown from the trees a cotton screen was built on three sides and about one foot from the ground to provide a free circulation of air. One mite was transferred to each cell immediately upon emerging from the egg and reared to the adult stage.

The cells were examined daily, each change of development being recorded as it appeared. In order to obtain sufficient magnification it was necessary to use a binocular microscope with stand removed, and this necessarily made the work slow and tedious. Approximately 100 cells were used at each brood and it was possible to rear from 50 to 75 mites by this method. Newcomer and Yothers used the cells both for rearing the young mites and for mating the adults to obtain laying records, but in the writer's experiments, the cells proved to be entirely unsatisfactory for the latter purpose. As a rule the adults mated within the cells, and frequently the female oviposited two to four eggs, but then became restless and either escaped or became entangled in the sticky material. A rearing cell about one inch in diameter was substituted, but this proved little better than the smaller cell. A whole leaf was then given to each mated female, with a circle of tanglefoot at the base of the petiole to prevent escape. The majority of the females remained upon the leaf and apparently deposited their full complement of eggs. This method was, on the whole, quite satisfactory.

A diagram was made of both sides of the leaf with the principal veins sketched in, and the oviposition dates were obtained by daily observations, each egg as it was deposited being recorded and properly located on the diagram. From these records the hatching dates and thus the length of the incubation period were obtained.

On account of the possible early deposition of winter eggs, beginning with the third generation, instead of the females being restricted to the leaf, each was given the range of a short piece of spur. The tanglefoot, therefore, was put down from the leaf about two inches instead of around the leaf petiole. This appeared to parallel natural conditions for it gave the female an opportunity to deposit both summer and winter eggs. It should also be stated that all leaves in the cluster except the one on which the adult was placed, were previously removed and the short piece of spur gone over with a dry tooth brush to remove any possible old winter eggs or empty shells.

It has been stated by Newcomer and Yothers (11) that the individual females in the later generations lay either all summer eggs or all winter eggs, this conclusion being based on the fact that the eggs either all hatched or all failed to hatch during the current season. However, they apparently used the leaf cells throughout the season and the females had no opportunity of depositing winter eggs naturally upon the twigs. In any event, the writer's investigations in Nova Scotia did not support this hypothesis. Three females of the third generation which laid one winter egg each also deposited a number of summer eggs. In the fourth generation, 21 females out of a total of 35 laid both summer and winter eggs. On this basis 60% of the females of this generation laid both types of eggs.

In conjunction with the rearing work at the laboratory, frequent visits were made to various infested orchards scattered over the western portion of the Annapolis valley. This served as a check on the development of mites in the field in comparison with the rearing work.

Number of Generations

It has been stated by Newcomer and Yothers (11) that there are six complete generations and a partial seventh and eighth generation in Washington state; Garman (7) in the east suggests six generations in Connecticut, while Ross (13) considers there are at least six ge [illegible] n Europe, Massie (8) states: "The number of generat [illegible] s, but it is considered that there are at least four in [illegible] rägårdh (14) reports that there are four generations i [illegible]

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The results obtained from the study of the European red mite during 1930 and 1931 indicate that there are five generations each year in Nova Scotia—two complete and three partial. The adults of the first and second generations lay summer eggs. The females of the third generation deposit a small percentage of winter eggs while the remainder and much larger number are laid on the foliage as summer eggs. In the rearing cages only three eggs were deposited on the twigs as winter eggs, this being a very small proportion of the total deposited. From orchard observations it was evident that a much larger proportion was deposited as winter eggs by the females of the third generation. On July 31, 1930, when oviposition by the females of the latter generation was practically complete, counts were made, in an orchard, of the number of eggs on the leaves and on the twigs. These counts indicate that of a total of 3399 eggs, 3030 were on the leaves and 369 on the twigs, or 10.86% winter eggs. These results, though not strictly accurate owing to unavoidable factors in making the counts, are at least a fair representation of the proportion of winter eggs deposited by the females of the third generation. Eggs were also deposited by females of the same generation upon the fruit, principally about the calyx. These presumably were winter eggs as none hatched during the current season.

The adults of the fourth generation also deposited both summer and winter eggs. In this instance it was necessary to rely upon those deposited in the rearing cages to obtain the proportion of each, it being impossible to make accurate counts in the orchard. The percentages of winter and summer eggs deposited by the females of the fourth generation in 1930 were 45.2 and 54.8 respectively. In 1931, the percentages were 34.8 and 65.2 The eggs of the fifth generation were all deposited on the twigs as winter eggs. From the results of the rearing work over a two-year period it is evident that the eggs found on the limbs during the winter are an accumulation of those deposited by the females of the third, fourth, and fifth generations.

Overlapping of Generations

In Fig. 1, there is represented the seasonal history of the European red mite at Annapolis Royal during the years 1930 and 1931. The dark lines indicate the duration of the various generations, at the extreme left the date when the first larva emerged and at the right, when the last adult died. The extremes are necessarily represented in this chart; therefore, the actual number of mites overlapping is comparatively small. At no time during the season was there more than an overlapping of the latest part of one generation with the beginning of the following one. All field observations made in various parts of the Annapolis valley throughout the seasons of 1930 and 1931 indicated that there was no pronounced overlapping of the various generations. With a pest of this nature this is somewhat contrary not only to the general expectation, but also to the prevailing opinion.

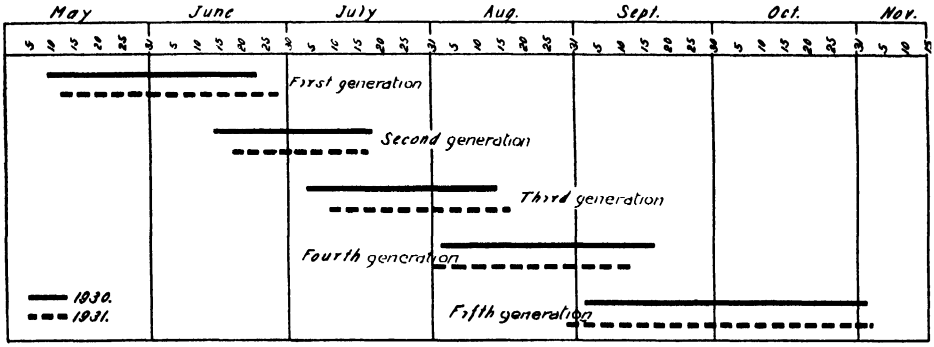

FIG. 1. *Seasonal history of the European red mite at Annapolis Royal, N.S., 1930 and 1931.*

Winter Eggs

The winter eggs are deposited upon the small outer branches and also on the larger limbs when these are free of loose scaly bark. They are largely laid on the under, or more protected, surface where they become massed in immense numbers and produce a red appearance which is distinguishable for some distance. A small proportion are also deposited upon the surface of the fruit more particularly about the calyx.

The eggs are bright red, maintaining their color throughout the winter without appreciable change until shortly before hatching when they turn somewhat paler. After hatching, the empty shells remain upon the limbs for some time and are silvery white and rather transparent. In form the egg is spherical, flattened at the poles, with fine ridges radiating from the visible pole. A short fine stalk projecting from this pole is visible only with a strong lens. In diameter the eggs vary from .152 to .162 mm. measured across the pole.

In 1930, the first winter egg was observed in the rearing cage on July 26, and in 1931, on July 28. In the orchard, winter eggs were laid at a slightly earlier date, the first being observed on July 22 in both years.

Under control, the last egg in 1930 was laid on October 27, and in 1931 on October 30. It is evident, therefore, that the winter eggs are deposited over a period of more than three months. It must be fully realized, however, that both of these seasons were exceptional since no frosts occurred until after the middle of October, some three to four weeks later than normal.

Summer Eggs

The summer eggs, as a rule, vary from a bright to a pale red. Some, however, are quite transparent with very little color. As the embryo develops, the eggs turn decidedly lighter in color and shortly before hatching have a rather watery, transparent appearance. In size they are slightly smaller than the winter eggs, varying from .145 to .160 mm. in diameter. In other respects the summer and winter eggs are similar.

The summer eggs are deposited on both surfaces of the leaves, with a somewhat larger portion on the under surface. A count of the eggs of the first generation on 86 leaves indicated 1505 eggs on the under surface and 1139 on the upper, or 57% and 43%. The larger number are laid along or near the midribs or larger veins.

The average incubation period of the eggs of the second, third and fourth generations in 1930 was between nine and ten days. Those of the fifth generation were about two days longer in hatching. In 1931, the eggs of the second and fifth generations hatched in approximately 13 days and those of the third and fourth in about nine days. In all generations there was some mortality of the summer eggs. This mortality, however, was too small to cause any noticeable suppression of the pest. The eggs which failed to hatch did not exceed 16% in any instance in the two years of investigation, and in the case of two of the 1930 generations were less than 8% of the total.

Adults

The newly emerged female is of a light shade of red, but within a few hours the color begins to turn darker and by the second day is dark red or chocolate brown. The body is full and decidedly rounded on the dorsum; the eyes are red.

The male is light yellow in color, but after feeding is usually greenish. The body is much smaller and more slender than that of the female and tapers sharply toward the caudal extremity. The eyes are red and prominent.

The adult male upon emerging is very active and soon seeks a quiescent female deutonymph. As a rule the male will remain in attendance until the female emerges, and it is not unusual for the male to wait in this manner from one to two days. Mating takes place immediately the female emerges, in fact the amorous male frequently assists the female to free herself from the moulting skin. A battle royal between the males is a common occurrence. This always proceeds until one is defeated and frequently more or less disabled. On one occasion the defeated male showed but little life for some time and after more than an hour could only feebly crawl.

The males are polygamous. A male in one instance mated with three females all within an hour. It was observed, however, that the last copulation period lasted only about two minutes, whereas the usual time was eight to twelve minutes. On another occasion a male mated twice during the afternoon and again with a virgin female the next morning. None of the females accepted more than one male.

After mating, the females feed on the leaves, extracting the leaf juices and depleting the foliage of chlorophyll or green coloring matter. The older leaves at the base of the current year's growth are preferred by the mites, and these as a rule are the first to lose the deep green and become pale and dull in color. As many as 140 adult females have been counted on these older leaves with only 5 to 20 at or near the terminal growth. Late in autumn, on sunny but otherwise cool days, the females congregate on the upper leaf surface where they remain for hours exposed to the sun's rays.

The adults were frequently observed crawling from a leaf by the way of the petiole to adjoining foliage. It appears, however, that this is, for the most part, the limit of movement.

The females in their movement over the limbs, in the process of laying winter eggs, spin some very fine silk. This webbing was only observed where the females were particularly numerous and it then extended along the limbs, over and about the massed eggs. Numerous eggs were found attached to this webbing. The silk was apparently easily removed from the trees by winds or storms for it disappeared soon after oviposition.

The length of life of the females and the number of eggs deposited vary with the different generations. For instance in 1931 the females of the second generation lived an average of 13.24 days with a maximum of 17 days. This was in contrast with an average of 24.25 days and maximum of 36 days for the females of the fifth generation. In 1930, the greatest number of eggs were deposited by the females of the second generation, and the smallest number by those of the fifth generation, the averages being 34.92 and 9.74 eggs respectively. Ordinarily, the pre-oviposition period was one to three days but this also varied somewhat with the different generations. The usual rate of egg deposition was one to as many as four eggs each day during a period of eight to ten days. There were days, however, when no eggs were deposited, this being observed more particularly with the fifth generation. Oviposition was observed at a temperature of 44° F. Virgin females oviposited and the eggs hatched but the resulting mites were all males. Whether these mites were capable of fertilizing females was not determined. Parthenogenesis has been recorded by Newcomer and Yothers (11), Banks (1), and McGregor and Newcomer (10).

The males are much more active than the females and move rapidly over the leaves. There was only a short period at the height of emergence when males were numerous, for after mating they disappeared quite rapidly, under natural conditions. In the rearing cages the males usually disappeared within from one to three days after mating. No accurate records, therefore, were obtained regarding the length of life of the adult males, but all evidence indicates a much shorter period than for the females.

Immature Forms

The immature or developing mite passes through three distinct stages or instars, known as larva, protonymph and deutonymph.

The larvae are readily distinguished from the other instars as they have only six legs. After the larvae moult there appears the full number of legs,– eight. Each instar is divided into two well defined periods, first an active feeding one, followed by a period of quiescence. The mite is dormant during the quiescent period. The legs are rigid, partly drawn under the body, whitish in color and rather transparent. Most of these quiescent forms are found on the under surface of the leaf. They also occur to some extent on the upper surface, and occasionally on the nearby fruit spurs. A few hours previous to moulting, the quiescent forms usually become quite silvery in appearance owing apparently to the outer skin becoming separated from the body within. When moulting, the skin splits across the back in order to allow the mite to escape. The cast skins are eventually blown away or washed off by rains, but they frequently accumulate and are quite conspicuous, particularly on the under surfaces of leaves.

The sexes are not easily distinguished until the last instar, when they can be separated quite readily. The deutonymph males are smaller and more slender than the females, and the abdomen of the male is more pointed posteriorly.

Upon hatching, the young larvae crawl to the limbs and soon start feeding on the tender leaves. They prefer to feed upon the under surface although in the orchard many are to be found on the upper surface. At the commencement of the rearing work many breeding cells were clipped to the upper leaf surface, but in every instance the mites became restless and wandered about the cell and finally escaped or became entangled in the sticky material. These cells were, therefore, discarded and all mites reared upon the under surface of the leaf. There was no extensive movement of the immature forms over the trees; in fact, there does not appear to be even migration from leaf to leaf unless there is an overcrowded condition. When this occurs there is a movement of the mites starting at or near the base of the current year's growth and proceeding toward the terminal. As they slowly proceed in this manner the leaves left behind show the characteristic mite injury of depleted chlorophyll and pale foliage.

The length of the larval-nymphal period varies somewhat with the different generations, that of the first and fifth being longer than the others. This has been largely attributed to the cooler weather prevailing in the spring and autumn. The length of the quiescent periods in the different instars is only slightly less than the active feeding period. This is shown in Table IV.

Hatching of the Winter Eggs

Daily records were taken of dates of hatching of the winter eggs of the European red mite for the seasons of 1930, 1931 and 1932 at Annapolis Royal. Hatching began in 1930 and 1931 on May 12, and in 1932 on May 15. This period of mite emergence lasted approximately two weeks. The weather has a marked effect upon emergence, for the hatching of the winter eggs in 1930 was practically suspended during cold, wet periods on May 15 and 16 and again on May 20 and 21. Similar results were recorded during 1931 and 1932. During these cold periods not only is hatching delayed but the young mites, already emerged, cling tenaciously to the foliage, in a more or less semi-dormant condition with little apparent feeding or development. This results in the later emerging mites catching up, so to speak, with those emerging earlier and the disparity in dates of emergence of adults is not as great as might be expected.

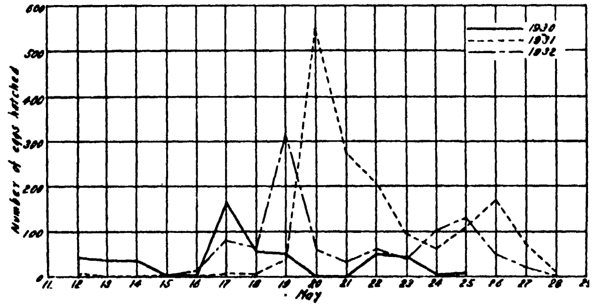

FIG. 2. *Diagram showing rate of hatching of winter eggs of the European red mite during the seasons of 1930, 1931 and 1932 at Annapolis Royal, N.S.*

Mortality of Overwintering Eggs

Data on the mortality of winter eggs were obtained in an orchard at Berwick in 1930 and in the insectary in 1930 and 1931. The insectary records were secured from twigs placed in vials containing water, some little time prior to egg hatching. The frequent handling of the twigs, in making daily counts of the emerging mites at the insectary, caused some injury to the eggs owing to their fragile nature. This has probably resulted in a somewhat higher mortality than ordinarily would occur. On the other hand, the direct orchard counts were not entirely satisfactory, as little control over natural factors seemed possible. The results are presented in Table III, but due allowance must be made for experimental error.

TABLE III

Mortality of winter eggs of the European red mite, 1930 and 1931

Year	Records at	Total no. eggs on twigs	Number eggs hatched	No. eggs failed to hatch	Mortality, %
1930	Insectary	1832	936	896	41.10
1930	Orchard	1740	1448	292	16.72
1931	Insectary	2286	1608	678	29.60

Immature Forms

Daily observations were made of the mites in the rearing cells and all changes in development recorded from the time of hatching of the winter eggs until the adults emerged. It has not been convenient to include in this paper the individual rearing records of the immature forms, owing to the space involved. A summary is, however, presented in Table IV, showing the average duration of the various periods in the development of the immature forms in 1930 and 1931.

TABLE IV

Comparison of feeding and quiescent periods of immature stages of the European red mite, Annapolis Royal, N.S.

Year	Generation	Larva		Protonymph		Deutonymph		Total average length immature period	
		Average feeding period, Days	Average quiescent period, Days	Average feeding period, Days	Average quiescent period, Days	Average feeding period, Days	Average quiescent period, Days	Feeding, Days	Quiescent, Days
1930	First	3.50	4.15	2.91	3.03	2.24	1.53	8.65	8.71
	Second	1.89	1.45	1.57	1.41	1.36	1.51	4.82	4.37
	Third	1.94	1.45	1.74	1.41	1.96	1.63	5.64	4.49
	Fourth	2.26	1.90	1.76	1.71	2.00	1.88	6.02	5.49
	Fifth	2.50	2.31	2.75	2.53	2.61	2.57	7.86	7.41
1931	First	2.71	2.66	1.86	2.10	1.57	2.37	6.14	7.13
	Second	2.21	1.93	2.20	1.71	2.19	1.57	6.60	5.21
	Third	2.31	1.70	1.98	1.77	1.83	1.45	6.12	4.92
	Fourth	2.40	1.47	2.01	1.84	2.37	1.88	6.78	5.19
	Fifth	3.28	2.43	2.41	2.26	3.31	3.31	9.00	8.00

Oviposition and Hatching of the Summer Eggs

In Fig. 3 are shown the periods at which oviposition and hatching of the different generations of the European red mite occurred in 1930 and 1931. A comparison of the laying and hatching dates shows uniformity for those two years. This was probably due, in a large measure, to the similarity of the seasons 1930 and 1931; both opened earlier than normal, and at about the same dates. The autumn in both years continued much milder than the average season, with no severe frosts at Annapolis Royal, until October 20. The average mean temperature was, of course, several degrees lower at the time of the fifth or last generation than earlier in the season; and it will be noted that the laying and hatching periods are more drawn out than those of the other generations.

Incubation of the Summer Eggs

The length of the incubation period varies somewhat with the different generations. This fluctuation is apparently largely due to the temperature factor, the midsummer generation showing the shortest period of incubation, with the last or autumn generation being several days longer. In Fig. 4 is shown the length of the incubation period of the summer eggs for all generations in 1930 and 1931.

In order to bring them together in condensed form, there is presented in Tables Vand VI a summary of the more important phases in the life history of the European red mite, for the years 1930 and 1931. In the first generation, the incubation period is of necessity not included in the seasonal averages as the young mites of this generation emerge from the winter eggs. The average length of the complete life cycle of the first generation is therefore reduced accordingly. The complete life cycle is several days longer in the first and fifth generations than in those intervening. The oviposition period is also of longer duration early and late in the season. The number of eggs deposited is greatest in the mid-season generations.

TABLE V

SUMMARY OF THE PHASES IN THE LIFE OF THE EUROPEAN RED MITE, ANNAPOLIS ROYAL, N.S., 1930

Generation	Average length of stages		Pre-oviposition period, Days	Average length of complete life cycle, Days	Average length of oviposition period, Days	Average number of eggs laid by each female	Average length of life of female, Days
	Incubation, Days	Immature forms, Days					
First		17.62	1.73	19.35	7.66	22.16	11.75
Second	9.21	9.20	2.18	20.59	10.66	34.92	14.53
Third	9.53	10.10	2.34	21.97	9.57	29.22	12.61
Fourth	9.81	11.45	3.22	24.48	10.62	17.65	16.20
Fifth	11.49	15.07	3.79	30.35	10.71	9.74	16.74
Average	10.01	12.69	2.65	23.35	9.84	22.74	14.36

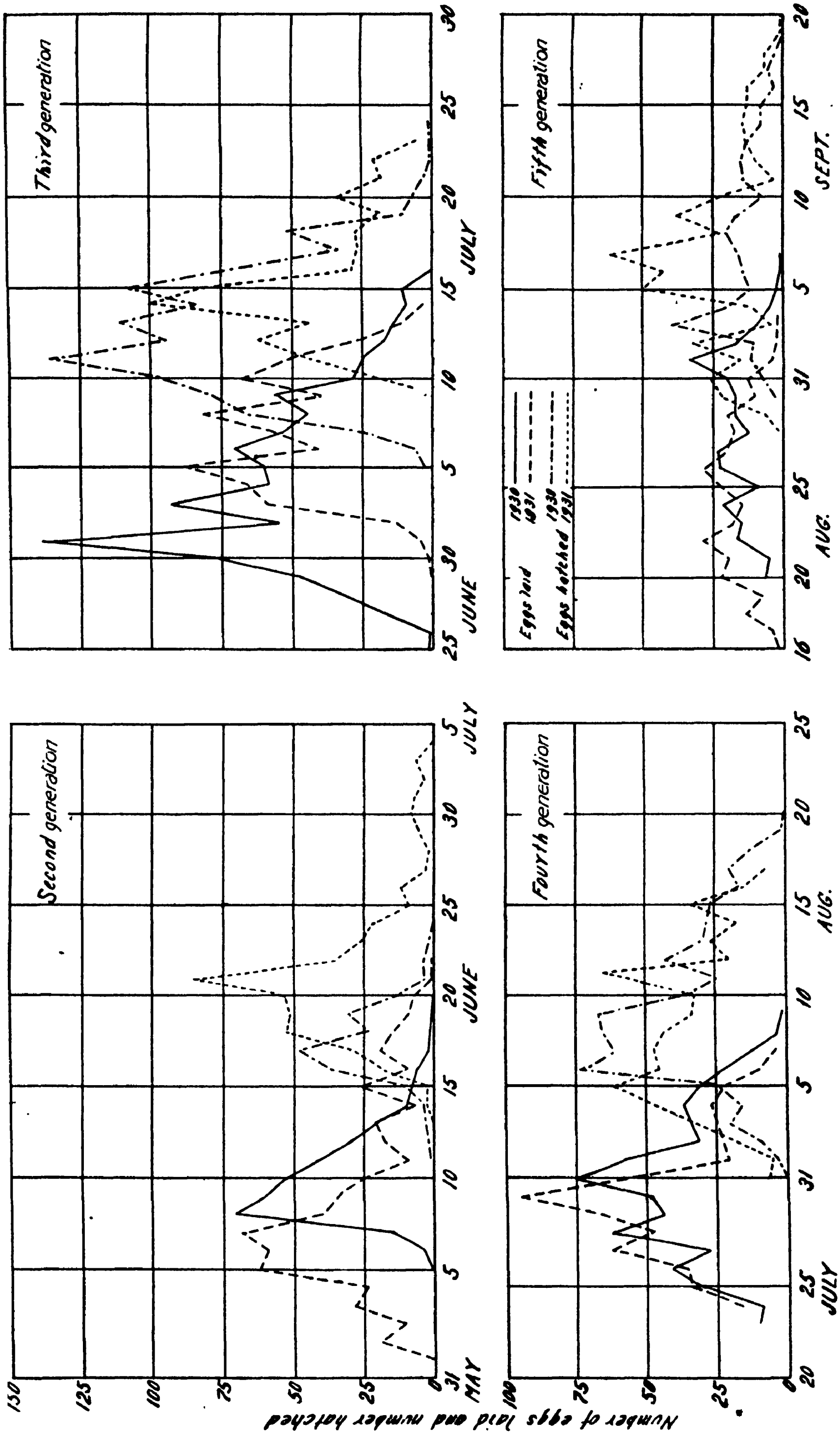

FIG. 3. Diagram showing rate of egg deposition and rate of hatching of generations of the European red mite during the seasons of 1930 and 1931, at Annapolis Royal, Nova Scotia.

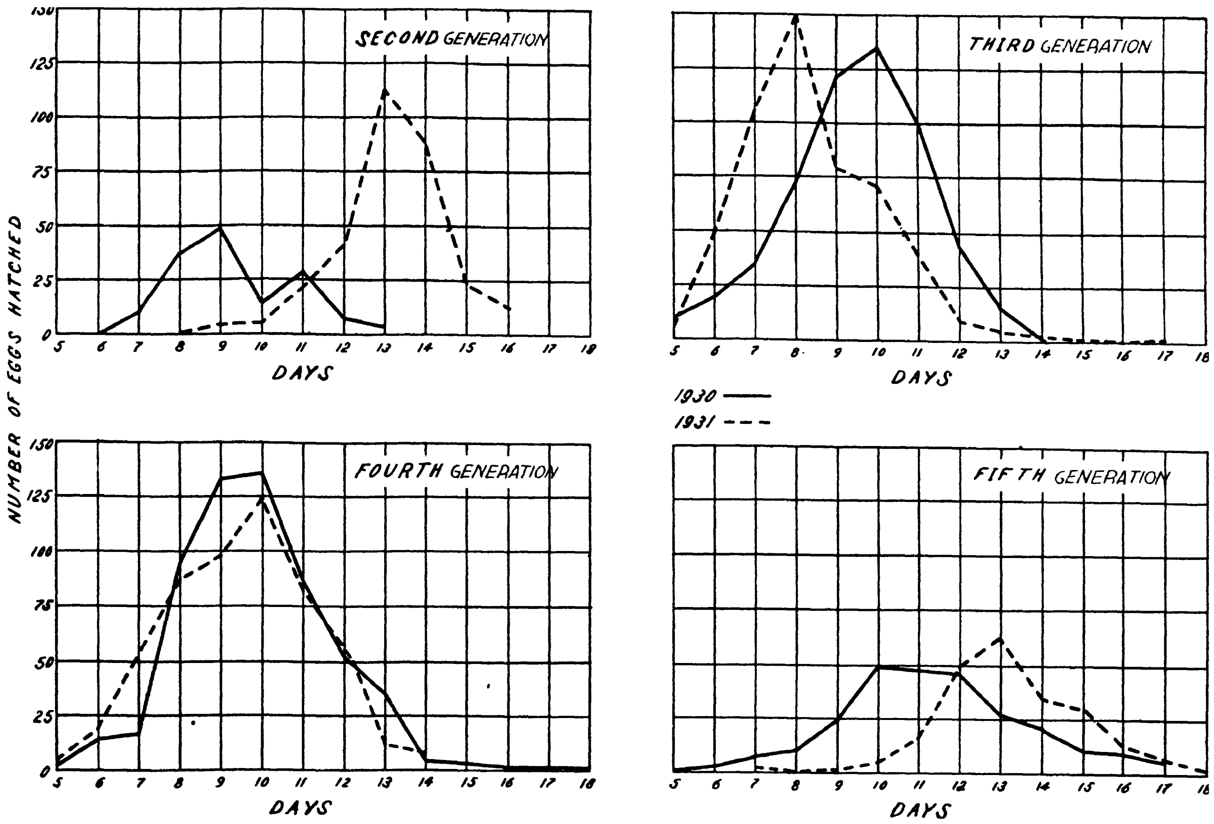

FIG. 4. *Diagram showing length of incubation period of generations of the European red mite for the seasons of 1930 and 1931, Annapolis Royal, Nova Scotia.**

TABLE VI

SUMMARY OF THE PHASES IN THE LIFE OF THE EUROPEAN RED MITE, ANNAPOLIS ROYAL, N.S., 1931

Generation	Average length of stages		Pre-oviposition period, Days	Average length of complete life cycle, Days	Average length of oviposition period, Days	Average number of eggs laid by each female	Average length of life of female, Days
	Incubation, Days	Immature forms, Days					
First		13.20	2.75	15.95	9.88	24.00	13.20
Second	13.14	11.77	2.93	27.74	8.90	27.20	13.24
Third	8.40	11.10	2.00	21.50	9.00	28.58	13.61
Fourth	9.49	11.97	4.22	25.68	9.55	19.63	16.32
Fifth	13.03	16.90	7.16	37.09	13.37	6.63	24.25
Average	11.01	12.98	3.81	25.59	10.14	21.20	16.12

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SOME PREDATORS OF THE EUROPEAN RED MITE, *PARATETRANYCHUS PILOSUS* C. & F., IN NOVA SCOTIA[1]

BY F. C. GILLIATT[2]

Abstract

Predators are the most important natural enemies of the European red mite. Notes are given on the life history and habits of the following predaceous enemies of this mite, *Seiulus pomi* Parrott; *Diaphnidia pellucida* Uhl.; *Diaphnidia capitata* Van D.; *Hyaliodes vitripennis* Say; *Stethorus punctum* Leconte; *Plagiognathus obscurus* Uhl.; *Camptobrochis nebulosus* Uhl.; *Anystis agilis* Banks; *Campylomma verbasci* Mey., and an unidentified species of Syrphidae.

During the summer of 1932 a study was made of the factors pertaining to the natural control of the European red mite in Nova Scotia. At the outset, the writer wishes to state that such a study leads one into byways and side issues, from which it is difficult to become extricated, and in the final analysis the conclusions may not be too clearly defined.

As far as can be determined, the most important natural control factors, relating to this orchard pest, are the numerous predaceous enemies. At no time were internal parasites observed. The known predators are varied and include species representing one order of the class Arachnida and four orders of the class Hexapoda. Those belonging to the first class are two species of the order Acarina, *viz.*:—*Seiulus pomi* Parrott and *Anystis agilis* Banks. Of the second class there are six species of the order Hemiptera, *viz.*:—*Diaphnidia pellucida* Uhl., *Diaphnidia capitata* Van D., *Hyaliodes vitripennis* Say, *Plagiognathus obscurus* Uhl., *Camptobrochis nebulosus* Uhl., *Campylomma verbasci* Mey. (all Miridae). There was a species of Coleoptera, *Stethorus punctum* Lec. The Diptera represented was an unidentified species of the family Syrphidae. There was one unidentified species of the order Thysanoptera.

The more important of these predators were so difficult to rear under controlled conditions that all such work was largely abandoned. No doubt they could have been artificially reared by devising special technique, but this would have rendered it impossible to deal at any time with more than a small number, thus limiting the viewpoint in general. The results of the investigation, therefore, are largely based on field observations, supplemented to some extent by certain artificial control work using large field cages.

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In the orchards examined there were always one or more species of predators feeding upon the European red mite. In some instances the predators were apparently few in number, while in many others they were numerous. It must be emphasized that it was not a simple matter in all instances to evaluate the various species of predators as far as numbers were concerned, for the reason that many are very minute and not readily observed without frequent, close and painstaking observations. Further, the various predators do not all appear at the same period during the summer. One may be disappearing when another is becoming abundant. There were periods between the generations of the predaceous mite *Seiulus pomi* Parrott when but few of the active forms were present, particularly during the first half of the season before there was any overlapping of the broods. As a result, in many instances, there were gaps during the season when predators were apparently not numerous and difficult to find. When these gaps occur for any extended period the pest rapidly increases in numbers owing to its great powers of reproduction. It is not likely that any one of the predators, acting alone, will bring about a full measure of control of the mites. Certainly the best results are quite apparent where two or more are present. This, it might be stated, is usually what occurs and there is, as a rule, a very definite increase of all the predators as soon as the mites begin to appear in large numbers.

The writer does not wish to infer that there are no other natural control factors except predators. There doubtless are others, and this was considered when the decline of the pest did not seem to be consistent with the number of predaceous enemies present. It may be further stated that this was the most confusing part of the investigation, and, at the close of the season, was not entirely solved to the satisfaction of the writer.

There is another point which must now be considered. At certain stages in the lives of many of the predators large numbers are killed by the various sprays ordinarily applied for the control of fungus diseases and insect pests. This is described later in detail, but in a general way it may be mentioned that all the Miridae in the nymphal stages are quickly killed with nicotine spray; oils and some of the ordinary fungicides seem to affect seriously the number of *Seiulus pomi* Parrott, particularly in the immature and adult stages. This explains the rapid increase of the European red mite after application of oils.

Again, lime-sulphur sprays have a repressing effect upon the European red mite and such sprays ordinarily prevent this pest from assuming outbreak form. On the other hand no such results are in evidence following the use of Bordeaux mixture. In fact it has been shown that one of the most important predators, *Seiulus pomi* Parrott, was seriously reduced in numbers by Bordeaux spray. When a grower changes from a sulphur spray program to one of Bordeaux mixture, the European red mite frequently assumes outbreak form at the end of the first season following the use of Bordeaux spray. The only explanation that can be given is that up to the time of the change, the sulphur sprays kept the red mite under control, and with the change to Bordeaux mixture the controlling factor was removed, with the result that the mite increased more rapidly than the predaceous enemies were capable of doing. In the orchards under special observation, the population of European red mite has been greatest on those areas treated with Bordeaux mixture, but orchards treated with sulphur sprays are, nevertheless, frequently subject to severe infestation.

All this, however, may be somewhat modified by various environmental conditions. The mite flourishes best in vigorous orchards and poorly cared for orchards of low vigor are not likely to show outbreaks of great degree. Again, when there are solid blocks of susceptible varieties, outbreaks are more liable to develop.

As a summary, the many predators must be considered in the light of a common enemy to the European red mite, a natural balance being established under normal environment. When there is interference with the factors of natural control there follows a rise in numbers of the pest which may end in destructive outbreaks.

Sprayed and Untreated Apple Trees in Relation to Prevalence of European Red Mite

It has been observed generally, that the European red mite appeared to increase in orchards sprayed with Bordeaux mixture in comparison with those treated with sulphur sprays of various kinds, or even in untreated orchards. All sulphur sprays are known to have a repressing influence upon red mite, while Bordeaux mixture does not have this effect. It also appears that natural control is influenced in some way by the use of Bordeaux mixture, probably as a result of the destruction or repulsion of the various predators that attack the mites. In England the European red mite has also been observed to increase more rapidly in Bordeaux sprayed orchards in comparison with those not so treated, and the increase has been attributed to a detrimental effect of the Bordeaux upon some natural control factor.

For the purpose of making regular counts of red mite throughout the season of 1931, an experimental orchard that had been treated with various standard sprays, was selected. Each plot in this orchard had received the same treatment for the preceding three years and a fair degree of European red mite infestation had been present for some time. The variety was Golden Russet, one of those susceptible to attack. There were five plots, four of which were treated with standard sprays and one check as follows: Bordeaux; lime-sulphur; lime-sulphur–iron sulphate mixture; lime-sulphur–aluminium sulphate mixture, and check plots. Counts of red mite were made every two weeks, or as near such dates as convenient. All counts were based on the number of mites, including all stages, per hundred leaves.

Table I fully explains the results of these counts. It will be observed that the Bordeaux treated plot at all times led the list in number of mites, by a wide margin. Early in the season lime-sulphur–iron sulphate mixture gave the least number of mites, but its place was soon taken by the straight lime-sulphur. Regarding the check, a word of explanation should be made. The

TABLE I

EUROPEAN RED MITE COUNTS FROM STANDARD SPRAY PLOTS, BERWICK, N.S., 1931

Date	Spray	Number of mites per 100 leaves			
		Adults	Eggs	Immature forms	Total, all stages
May 29	Bordeaux	0	0	169	169
29	Lime-sulphur	0	0	54	54
29	Lime-sulphur–iron sulphate	0	0	16	16
29	Lime-sulphur–alum. sulphate	0	0	41	41
29	Check	6	0	78	84
June 12	Bordeaux	29	1131	0	1160
12	Lime-sulphur	4	153	0	157
12	Lime-sulphur–iron sulphate	0	49	0	49
12	Lime-sulphur–alum. sulphate	3	124	0	127
12	Check	15	395	0	410
June 25	Bordeaux	7	422	732	1161
25	Lime-sulphur	0	12	4	16
25	Lime-sulphur–iron sulphate	0	42	9	51
25	Lime-sulphur–alum. sulphate	1	40	43	84
25	Check	6	215	166	387
July 9	Bordeaux	84	2011	15	2110
9	Lime-sulphur	5	174	2	181
9	Lime-sulphur–iron sulphate	7	70	0	77
9	Lime-sulphur–alum. sulphate	19	181	12	212
9	Check	84	1292	88	1464
July 28	Bordeaux	284	1558	310	2152
28	Lime-sulphur	15	32	20	67
28	Lime-sulphur–iron sulphate	15	127	10	152
28	Lime-sulphur–alum. sulphate	46	113	54	213
28	Check	73	298	86	457
Aug. 12	Bordeaux	130	2262	920	3312
12	Lime-sulphur	32	432	78	542
12	Lime-sulphur–iron sulphate	56	346	72	474
12	Lime-sulphur–alum. sulphate	42	486	192	720
12	Check	48	880	270	1198
Aug. 31	Bordeaux	220	2644	502	3366
31	Lime-sulphur	20	114	20	154
31	Lime-sulphur–iron sulphate	56	1038	110	1204
31	Lime-sulphur–alum. sulphate	34	488	48	570
31	Check	28	230	46	304
Sept. 16	Bordeaux	394	86	626	1106
16	Lime-sulphur	50	38	92	180
16	Lime-sulphur–iron sulphate	182	38	300	520
16	Lime-sulphur–alum. sulphate	38	22	120	180
16	Check	12	14	18	44
Sept. 29	Bordeaux	216	44	148	408
29	Lime-sulphur	54	0	4	58
29	Lime-sulphur–iron sulphate	184	10	80	274
29	Lime-sulphur–alum. sulphate	62	4	32	98
29	Check	26	0	6	32

TABLE I—(*Concluded*)

EUROPEAN RED MITE COUNTS FROM STANDARD SPRAY PLOTS, BERWICK, N.S., 1931—*Concluded*

Date	Spray	Number of mites per 100 leaves			
		Adults	Eggs	Immature forms	Total, all stages
Oct. 19	Bordeaux	64	0	8	72
19	Lime-sulphur	26	0	2	28
19	Lime-sulphur–iron sulphate	24	2	1	27
19	Lime-sulphur–alum. sulphate	24	5	2	31
19	Check	0	0	0	0
Nov. 3	Bordeaux	80	0	4	84
3	Lime-sulphur	36	0	0	36
3	Lime-sulphur–iron sulphate	38	0	0	38
3	Lime-sulphur–alum. sulphate	34	0	0	34
3	No leaves on check plots				
		Winter eggs on limbs			
Nov. 12	Bordeaux	3035			3035
12	Lime-sulphur	187			187
12	Lime-sulphur–iron sulphate	658			658
12	Lime-sulphur–alum. sulphate	464			464
12	Check	437			437

foliage was badly affected with scab and after midsummer the leaves were cracked, with many dead areas, and were harsh and dry and not nearly so attractive to the mites as the clean, sprayed foliage on the treated plots. No matter what other factors may be present, scabby foliage does not afford the same favorable conditions for red mite as sprayed, healthy foliage, and, if not the only factor, is certainly an important reason why untreated orchards are seldom severely attacked. By October 15, all the foliage had dropped from the check trees and a much larger percentage of the winter eggs must have been laid by the third and fourth generation females here in comparison with those on the sprayed plots.

It will be observed from the table that the mite counts on November 3 were higher than on October 19. On November 3, at least 90% of the foliage had turned yellow, and the red mite always deserts the yellow and migrates to the green, healthy leaves. At the former date the only green foliage on the treated plots was one to three leaves of the terminal growth, all of the remainder having turned yellow, although only a few had fallen. All the adult females had concentrated on these green leaves. When mite counts were made on November 3, only these green leaves were selected. This resulted in a higher mite count than would have occurred had the mites been distributed over the trees.

In making counts of winter eggs on these plots, difficulty was experienced in devising a method that would be accurate. However, terminal twigs were selected, eliminating the current year's growth, ten representative twigs being taken from each plot. These were measured six inches back of the current year's growth and the winter eggs counted on the measured portion. This gave sixty inches of limb from each plot. Comparing one plot with another, this method gave fairly accurate results.

The results as shown in Table I indicate what can be expected from the use of these standard sprays, as far as red mite is concerned. Observations made at various times in other infested orchards helped further to corroborate these results. Of the sulphur sprays the straight lime-sulphur was the most effective in holding the red mite in check. This was followed by aluminium sulphate mixture, with iron sulphate mixture the least effective. Bordeaux without doubt promoted the increase of red mite in some manner, over either sprays or check. The check had, next to the lime-sulphur plot, the smallest number of winter eggs. With such severe scab infection on the check trees as occurred in 1932, a low mite count was almost inevitable. Although the various predators were more in evidence throughout the season on the check than on the treated plots, it is quite conceivable that a greater mite population would have occurred had there been only a slight or moderate infection of leaf scab.

The following are the individual predators that have been observed attacking the European red mite, with their relation to the economic status of this pest in Nova Scotia apple orchards.

Seiulus pomi Parrott

This mite is probably the most important of all the predators attacking the European red mite in Nova Scotia orchards, not that it consumed more mites during the many periods under observation in comparison with other predators, but on account of its being active for the entire season from early spring before red mite eggs hatch, until well after all winter eggs have been deposited. This predator was also found more generally disseminated than others. The predaceous habits of *Seiulus pomi* Parrott are emphasized by Newcomer and Yothers (1), who observed that the European red mite eggs, and occasionally the immature forms, were consumed, but further stated that adults were not attacked. The writer has observed the adults and more mature nymphal stages of *Seiulus pomi* Parrott attacking all stages of *Paratetranychus pilosus* C. and F. Eggs probably represent the larger proportion of the total consumed, but the immature forms, more particularly during the quiescent periods, were readily attacked. It was also a common occurrence to observe adults, both male and female, being seized and the contents of the body emptied.

The adults of *Seiulus pomi* Parrott hibernate during the winter on the twigs, concealed in such places as under rough bark, in old bud-moth hibernacula and even under deserted oyster shell scales. The mites are active at rather cool temperatures and consume the European red mite winter eggs on the warmest days until late in the autumn. Feeding is again resumed in the spring, a considerable time before the mite eggs hatch. In fact, it may be that a limited amount of feeding upon the eggs may occur during the warmest days in the winter. The hibernating mites on cold winter days become practically as active as during the summer if brought to the laboratory and placed for a few minutes in a warm room. At such times, however, actual feeding on the eggs of the European red mite was not observed.

Table II, giving counts of winter eggs of red mite, will suggest just what reduction of living eggs takes place between the autumn and the following spring. The method of selecting branches for winter egg counts was similar to that already described. The autumn and spring counts were made from twigs taken from the same trees in the various plots and in as like manner as possible. The sucked out eggs are white and can readily be distinguished from normal healthy ones. There was some chance for error in making the winter egg counts, but there is a wide difference in the number of normal eggs in the autumn and in the spring.

TABLE II

NUMBERS OF EGGS OF EUROPEAN RED MITE ON APPLE TWIGS IN AUTUMN OF 1931 AND SPRING OF 1932, BERWICK, N.S.

Summer treatment	Date of counts	Number of normal eggs	Percentage reduction
Bordeaux spray	Nov. 11/31	3035	
	May 12/32	599	80.3
Lime-sulphur–spray	Nov. 11/31	187	
	May 12/32	87	53.5
Lime-sulphur–iron sulphate	Nov. 11/31	658	
	May 12/32	344	47.7
Lime-sulphur–aluminium sulphate	Nov. 11/31	464	
	May 12/32	189	59.1
Check	Nov. 11/31	437	
	May 12/32	86	80.2

Many factors in addition to predators probably contribute toward this reduction of winter eggs, *e.g.*, fungus diseases, heavy winds, frosts, sleet storms, desiccation, extreme low temperatures with varying climatic conditions, and possibly small birds. From observations made during the past two years it would appear that predators are the most important, and that *Seiulus pomi* Parrott is one of the most efficient of these in the Annapolis valley orchards.

An attempt was made to rear *Seiulus pomi* Parrott on potted apple seedlings in order to obtain at least a general life history and further details of the habits of this predaceous mite. It did not, however, adapt itself to such artificial conditions and this method out of necessity was abandoned. A low growing apple tree was partially caged with cotton and individual limbs used for life history observations. Apparently there are three generations of *Seiulus pomi* Parrott under the climatic conditions of Nova Scotia.

In 1932, the eggs of the first generation were deposited by the hibernating females beginning the last week of May. Eggs were most numerous during the first week of June. The last or third generation eggs reached maximum numbers during the last days of August. The last egg was observed on September 12. The second or intervening generation is approximately midway between the first and third generations. The incubation period varied from 7 to 10 days. It was not definitely determined just how many eggs each female deposited, but the general range was from 6 to 12, in any event not a large number.

The eggs are pearly white in color, ovate and very small, being only .062 mm. at the greatest, and .052 mm. across the smallest diameter. They are deposited on the undersides of the leaves near the midrib or larger veins. Apparently they are not attached to the leaf, but held in place by the leaf hairs.

As a rule the adults of *Seiulus pomi* Parrott are pale without characteristic markings. An occasional individual with pale brownish markings on the dorsum, and a few with the dorsum a pale reddish or wine color were observed. The latter coloration was apparently due to the mites gorging themselves on European red mite eggs, the color showing through the integument. The adults are more frequently found near the midrib and usually on the under surface of the leaf. They give the impression of sluggishness, but when disturbed they move rapidly over the leaf. The main difference in appearance between the adult and young mites is in size, although the latter are usually paler, being practically white in the younger stages.

It was possible to rear *Seiulus pomi* on foliage in the absence of the European red mite, the leaf juices being their only food. The species is, therefore, not entirely carnivorous. A special effort was made to obtain data relative to the rate of consumption of red mites by this predator. The results are given in Table III:—

TABLE III

RATE OF CONSUMPTION OF EUROPEAN RED MITE, IMMATURE FORMS, BY THE OVERWINTERING *Seiulus pomi* PARROTT ADULTS

Date	Number of *S. pomi* on each spur	Number of *P. pilosus* nymphs on each spur	Number of nymphs after ten days	Reduction, %	Average number of immature forms consumed per day
May 20	2	90	25		
23	1	16	2		
23	3	32	7		
26	2	45	7		
26	3	25	1		
27	2	115	40		
27	1	39	12		
28	1	24	10		
30	2	30	9		
30	2	85	25		
		501	148	72.45	1.91
May 25	Check	74	73		
25	Check	77	75		
30	Check	22	22		

In this experiment the average consumption per day by each adult *Seiulus pomi* Parrott was 1.91 European red mites. This was a reduction over the ten day period of 72.45%. At this rate about 26 immature forms of the first brood would be consumed by each predator. This is considering a period of only 14 days which was the average duration of the immature stage of the European red mite at this season. Actually the period would extend to 20–25 days as all do not mature at the same time and the number of mites consumed by each predator would be about 40 rather than 26. Of course, the percentage of mites consumed in a given feeding experiment would vary according to the number present. In the event of the European red mites being greatly increased in proportion to the predaceous mites, the percentage consumed would be decreased accordingly. It would seem, however, that the average rate of consumption per day should remain at least fairly constant.

Seiulus pomi Parrott was found in all infested orchards examined during 1932. In some the number was not great while in others they were relatively numerous. Table IV shows the results of leaf counts made in various orchards to obtain the number of *Seiulus pomi* Parrott per leaf.

TABLE IV

AVERAGE NUMBER OF THE PREDACEOUS MITE, *Seiulus pomi* PARROTT, PER LEAF IN ORCHARDS IN VARIOUS LOCALITIES IN THE ANNAPOLIS VALLEY, 1932

Date of count	Orchard number	Number of leaves counted	Number of *S. pomi*	Average per leaf
Aug. 30	1	100	445	4.45
Sept. 8	2	100	102	1.02
8	3	100	26	0.26
14	4	100	210	2.10
14	5	100	210	2.10
30	6	100	67	0.67
30	7	100	153	1.53
30	8	100	373	3.73
30	9	100	185	1.85

Further feeding experiments were conducted by placing a certain number of adult predaceous mites and newly emerged female European red mites on individual apple spurs. From this experiment there was obtained a fairly accurate indication of the number of European red mite eggs present on the foliage after a period of 10 days, with an association of predator and host *versus* an equal number of female European red mites but no predator. The results of this feeding are presented in Table V.

In most orchards there appeared to be a direct relation between the numbers of predaceous mites and of the European red mite. This apparently occurred except where the orchard practice has been such as to affect directly or to prevent this approximate balance between host and predator. It is certain that some of the sprays ordinarily used in the Annapolis valley, not only control injurious pests but at the same time kill many beneficial predators. As a result the natural balance is thrown out of adjustment in favor of the pest.

TABLE V

AVERAGE NUMBER OF RED MITE EGGS ON FRUIT SPURS IN THE PRESENCE AND ABSENCE OF PREDACEOUS MITES AFTER A 10 DAY PERIOD, ANNAPOLIS ROYAL, N.S., 1932

Date	Number of *S. pomi* adults on each spur	Number of adult females of *P. pilosus* on each spur	Number of eggs of *P. pilosus* after ten days	Average number of eggs of *P. pilosus*	Per cent reduction eggs over check
June 8	2	7	132	16.6	
8	2	7	148		
8	2	7	124		
8	2	7	39		
8	2	7	146		
8	Check	7	267	36.7	54.7
8	Check	7	256		
8	Check	7	249		
13	7	44	972	20.1	
13	Check	44	1563	35.5	37.7

A concrete example may be cited. Dormant oil spray not only destroys most of the winter eggs of the European red mite, but also kills the larger proportion of the hibernating adults of *Seiulus pomi* Parrott, thus largely removing probably the most important natural controlling factor. It follows, therefore, that when dormant oil sprays have once been resorted to in controlling European red mite, they must, as a rule, be applied about every two years as the mites increase again very rapidly, the natural control apparently being so reduced as to be ineffective.

A test of the various standard sprays was made to determine to some extent, the effect of such sprays on *Seiulus pomi* Parrott. This was done by transferring the mites to cells one inch in diameter clipped to a leaf. Results are presented in Table VI.

TABLE VI

RESULTS OF VARIOUS SPRAYS ON THE PREDACEOUS MITE, *Seiulus pomi* PARROTT, ANNAPOLIS ROYAL, N.S., 1932

Date	Number of adults	Spray	Number dead in 5 hr.	Number dead in 24 hr.	Number dead in 48 hr.	Remarks
Oct. 1	30	3% oil in 3 : 10 : 100 Bordeaux	20	30		
6	30	Bordeaux 3 : 10 : 100 plus 1 lb. arsenate lime	30			
6	39	Lime-sulphur 1 : 40, arsenate lead, 1 : 40	0	2	10	
6	15	Check	0	0	0	
7	32	Nicotine 1 : 800	0	0	0	
8	30	Standard lime-sulphur, iron sulphate mixture	25	26	26	Live mites feeble after 24 hr.
8	26	Standard lime-sulphur, aluminium sulphate mixture	15	26		
8	20	3% Sunoco oil	10	15	20	
Dec. 29	25	3% Sunoco oil	10	25		

In some respects this test must be considered as severe, for not only were the mites well covered with spray but they were actually immersed for some time in the solution as it more or less filled the cell. Nicotine evidently has no detrimental effect upon this predator, and lime-sulphur was the only spray that did not completely destroy the mites eventually. Ordinarily actual spraying would not so thoroughly cover such a large percentage of these predators as in the above experiment, therefore it is doubtful if more than a small proportion of the predators would be killed in the application of lime-sulphur. Further, when oils are applied in the dormant season many of the predators would be more or less concealed as already mentioned and 100% contact could not be expected.

We would not like to consider these results conclusive as the test was entirely too limited. However, the results are suggestive and indicate the probable effect upon this predator, of sprays ordinarily used in Annapolis valley orchards.

Diaphnidia pellucida Uhl.

This mirid, identified in 1930, was one of the first predators to be observed attacking the European red mite in Nova Scotia orchards.

In both the nymphal and adult stages it was very active and fed upon all stages of the mites. It was interesting to observe the movements of these insects when searching for their host. They did not search promiscuously but were very methodical, proceeding slowly and deliberately over the leaf surface using their beaks to search for prey. The beak was moved rapidly from side to side between the front pair of legs, completely covering a portion of leaf about $\frac{1}{16}$-in. in width. By proceeding slowly in this manner in an irregular course over the leaf they located the various stages of the mites. The beak is an extremely sensitive organ by which all food is located. While actually searching for food as described their legs or other parts of the body frequently come in contact with a mite, but this in no way appeared to direct them toward their food. When capturing the immature forms, or an adult female mite, they become quite excited, moving quickly and skilfully. The host is held firmly with the front legs and the beak quickly inserted, withdrawing the contents of an egg is about one minute. It takes only slightly longer to empty the body of an adult mite. The beak is usually inserted only once in an egg but frequently is withdrawn from an adult and again inserted in another part of the body until there remains only the outer skin. The empty eggs at once become transparent but do not collapse, remaining on the leaf for some time in their original form. Owing to the greater activity of the male in comparison with the female mites, the former were only occasionally captured.

It was found so difficult to rear this capsid in confinement that this part of the investigation was practically abandoned. The nymphs soon died in the cages and the adults also lived but a short time and produced no eggs. The following life history notes were from necessity obtained entirely from field observations.

The winter is evidently passed in the egg stage, as the first appearance of the insect in 1932 was on June 15, when the newly hatched nymphs were observed. The duration of the nymphal period was about three weeks. It should be mentioned in this connection that stray nymphs and adults were seen on many occasions during August, and the latest observed was on September 14. No detailed comparisons were made between these late appearing individuals and those observed when the numbers were at a maximum during June and July, but a superficial examination revealed no difference. However, there is the possibility that another closely related species may be involved.

The predaceous habits of this insect were consistent, and in the aggregate large numbers of mites were consumed, the measure of control depending, of course, upon the numbers present. The period when most mites were consumed was from about June 15 to July 15 or possibly a little later. This predator was found to be well disseminated in most infested orchards and often sufficiently numerous to effect a great reduction of the pest. In other instances results were not so apparent owing to lack of numbers. Of the many predators attacking the European red mite in Nova Scotia, *Diaphnidia pellucida* Uhl. is one of the most important.

Table VII will convey some idea of the rate at which red mites are consumed by *Diaphnidia pellucida* Uhl.:—

TABLE VII

NUMBERS OF EUROPEAN RED MITES CONSUMED BY *Diaphnidia pellucida* UHL. IN NYMPHAL AND ADULT STAGES IN CONSECUTIVE HALF-HOUR PERIODS, ANNAPOLIS ROYAL, N.S., 1932

Date	Period of observation	Predator	Numbers consumed				
			Eggs	Immature forms	Adult female	Adult male	Total
July 15	1st half hr.	Nymph	3	3	2	1	9
	2nd half hr.	Nymph	4	3	1	2	10
	3rd half hr.	Nymph	2	4	0	0	6
	4th half hr.	Nymph	3	2	2	0	7
			Total consumed in 2 hr.				32
	1st half hr.	Adult	5	5	3	1	14
	2nd half hr.	Adult	6	3	3	0	12
	3rd half hr.	Adult	6	4	4	1	15
	4th half hr.	Adult	5	3	2	0	10
			Total consumed in 2 hr.				51
Aug. 8	1st half hr.	Nymph	1	9	4	0	14
	2nd half hr.	Nymph	1	1	2	1	5
	3rd half hr.	Nymph	1	2	0	1	4
			Total consumed in 1½ hr.				23
	1st half hr.	Nymph	2	14	2	1	19
	2nd half hr.	Nymph	5	9	4	0	18
			Total consumed in 1 hr.				37

This predator, like *Seiulus pomi* Parrott, may be greatly reduced in numbers through the ordinary spraying operations. The nymphs are quickly killed with nicotine sprays. If, therefore, nicotine is included in the later sprays, large numbers of this predator are killed. It was also found that nicotine had little effect upon the adults. This was largely due to their habit of taking wing when the first spray hit the tree and thus avoiding contact. Even those receiving a mist were not killed, owing to the coating of fine hairs which prevents the wetting of the body. It was only with severe drenching that any mortality was observed, and this seemed to occur only on rare occasions.

Diaphnidia capitata **Van D.**

This species closely resembles *Diaphnidia pellucida* Uhl. in many respects. The nymphs of the two species are difficult to separate in the field. The adults, however, are easily distinguished as *capitata* is slightly smaller, and has a black head whereas in *Diaphnidia pellucida* Uhl. the head is pale greenish. Not a great deal is known of the life history of *Diaphnidia capitata* Van D., but as near as could be determined, the seasonal appearance of these two species was much the same. The nymphs of *Diaphnidia capitata* Van D. were first observed in 1932, on June 19, and the adults in the latter half of July. Both the nymphs and adults fed on all stages of the European red mite, the rate of consumption being much the same as with *Diaphnidia pellucida* Uhl. In Table VIII is shown the rate at which European red mites were consumed by the nymphs of *Diaphnidia capitata* Van D.

TABLE VIII

NUMBERS OF EUROPEAN RED MITES CONSUMED BY *Diaphnidia capitata* VAN D., IN CONSECUTIVE HALF-HOUR PERIODS, ANNAPOLIS ROYAL, N.S., 1932

Date	Predator	Time	Numbers consumed				
			Eggs	Immature forms	Adult female	Adult male	Total
July 15	1. Fifth instar	1st half hr.	1	7	1	0	9
		2nd half hr.	0	11	5	0	16
		3rd half hr.	2	5	3	0	10
		4th half hr.	4	4	4	0	12
							47

Diaphnidia capitata Van D. is of much less importance in the Annapolis valley than *Diaphnidia pellucida* Uhl. as it is less numerous. Although it was observed frequently at widely scattered points, in no instance were its numbers sufficient to cause any appreciable reduction in the European red mite population. Like *Diaphnidia pellucida* Uhl. the nymphs were quickly killed with nicotine spray.

Hyaliodes vitripennis **Say**

One of the common predators of the European red mite is the mirid, *Hyaliodes vitripennis* Say. This predator, in both nymphal and adult forms, was observed feeding upon all stages of the mite. The habits of this insect and its method of capturing and feeding upon mites were similar to those of the capsids which have been previously mentioned. It usually takes about one minute to empty an egg, and from one to two minutes to remove the body fluid of an adult mite. As a rule only the collapsed skin of the mite remains, but occasionally the body content is only partially removed, always resulting, however, in death to the mite. On one occasion a nearly mature nymph of *Hyaliodes vitripennis* Say attempted repeatedly to puncture with its beak an adult of the predaceous mite *Seiulus pomi* Parrott. This it was unable to do, as the dorsum of the mite proved too hard to be penetrated.

When the appetite of *Hyaliodes vitripennis* Say is temporarily satisfied, it spends considerable time at rest, on the leaf, cleaning or stroking its antennae and beak. This is done by seizing these organs with the front pair of legs and drawing them between for their entire length. The nymphs when moving over the leaf usually carry the caudal portion of the abdomen in a partially raised position, frequently nearly vertical.

In Table IX is shown the rate of consumption of all forms of the European red mite by the nymphs of *Hyaliodes vitripennis* Say:—

TABLE IX

NUMBERS OF EUROPEAN RED MITES CONSUMED BY *Hyaliodes vitripennis* SAY, IN CONSECUTIVE HALF-HOUR PERIODS, ANNAPOLIS ROYAL, N.S., 1932

Date	Predator	Time of observation	Numbers of mites consumed				
			Egg	Im-mature forms	Adult female	Adult male	Total
July 8	2nd instar	1st half hr.	3	7	0	2	12
		2nd half hr.	1	8	0	0	9
		3rd half hr.	0	3	0	0	3
							24
July 8	2nd instar	1st half hr.	0	8	0	1	9
		2nd half hr.	1	11	0	0	12
		3rd half hr.	0	3	0	1	4
							25
July 9	2nd instar	1st half hr.	1	10	0	3	14
		2nd half hr.	0	9	1	1	11
		3rd half hr.	0	2	2	0	4
							29
July 9	2nd instar	1st half hr.	0	10	0	3	13
		2nd half hr.	0	9	1	1	11
		3rd half hr.	1	1	0	0	2
							26
July 15	4th instar	1st half hr.	3	4	13	0	20
		2nd half hr.	3	0	5	0	8
		3rd half hr.	11	3	6	1	21
		4th half hr.	12	3	8	1	24
							73
July 15	3rd instar	1st half hr.	1	1	6	0	8
		2nd half hr.	3	0	6	1	10
		3rd half hr.	12	0	4	0	16
		4th half hr.	3	2	2	0	7
							41

The newly hatched nymphs of *Hyaliodes vitripennis* Say began to appear in orchards in 1932 during the last days of June. The nymphal period lasts for about four weeks. The first adult was recorded on July 25 and they were numerous during the first days of August. After August 7 they began to decrease rapidly in numbers. Although the eggs were not found, the fact that the nymphs appear upon the trees in the spring indicates that they must be deposited during the latter part of the summer, somewhere about the trees. There is only one brood in a season.

This bug is one of the most important enemies of the European red mite in the Annapolis valley. It is larger than the two capsids already discussed and consumes relatively more mites. Apparently the nymphs are somewhat more persistent in their predaceous habits than the adults. The latter are, however, predaceous feeders to a very considerable extent. It is unfortunate that the feeding period of this group of predators does not extend over a greater portion of the season. The period of activity of *Hyaliodes vitripennis* Say is from the last week of June to about the end of the first week of August, a period of about five to six weeks. During this part of the summer of 1932 many European red mite infestations were effectually held in check by this predator.

Nicotine was very toxic to the nymphs of this species also. The adults, owing to their activity escaped to a very large extent when the spray was applied. The application of sprays including nicotine after June 25 would destroy most of the nymphs of this predator.

Stethorus punctum Leconte

This is a small species of coccinellid beetle closely related to the western form *Stethorus picipes* Casey, which is an important predator of the European red mite in the Pacific Northwest.

On July 21, 1932, the larvae of *Stethorus punctum* Leconte were observed in a mite-infested orchard near Bridgetown, N.S. Observations were made for a few minutes only and no mite-feeding habits noticed. A week later its predaceous habits were observed. The larvae of the beetle crawl slowly over the leaf, devouring the mites as they come into contact with them. Eggs and the young mites were the forms chiefly consumed. As the larvae move comparatively slowly adult mites often escaped. However, adults occasionally were seized and devoured.

Table X shows the numbers of mites consumed by the beetles, during consecutive half-hour periods of observations.

Very little is known of the life history of this species of predator. When first observed on July 21 the larvae were more than half grown. Adults were first observed on July 28, and at that date there were about equal numbers of larvae and pupae. The adults are very small; those measured being from 1.3 to 1.5 mm. long. They are black with brownish legs, and the wing covers are clothed with short hairs. After the adults emerged they did not

TABLE X

NUMBERS OF EUROPEAN RED MITES CONSUMED BY *Stethorus punctum* LECONTE, IN CONSECUTIVE HALF-HOUR PERIODS

Date	*Stethorus punctum*	Time	Numbers of mites consumed				
			Eggs	Im-mature forms	Adults		Total
					Females	Males	
July 29	Larva	1st half hr.	0	8	0	0	8
		2nd half hr.	1	4	0	0	5
		3rd half hr.	3	1	0	0	4
		4th half hr.	0	5	0	0	5
		5th half hr.	2	5	0	0	7
		6th half hr.	1	1	0	0	2
			Total consumed in 3 hr.				31
July 29	Larva	1st half hr.	0	8	0	0	8
		2nd half hr.	2	4	0	0	6
		3rd half hr.	1	0	0	0	1
		4th half hr.	1	3	0	0	4
		5th half hr.	0	0	0	0	0
		6th half hr.	1	3	1	0	5
			Total consumed in 3 hr.				24
July 30	Adult	1st half hr.	5	8	1	1	15
		2nd half hr.	3	0	0	0	3
		3rd half hr.	1	1	1	0	3
		4th half hr.	3	4	1	0	8
			Total consumed in 2 hr.				29
Aug. 3	Adult	1st half hr.	1	10	0	0	11
		2nd half hr.	1	5	0	0	6
		3rd half hr.	1	1	0	0	2
		4th half hr.	0	5	1	0	6
			Total consumed in 2 hr.				25
Aug. 3	Adult	1st half hr.	0	5	1	0	6
		2nd half hr.	0	4	1	0	5
		3rd half hr.	0	1	0	0	1
			Total consumed in 1½ hr.				12
Aug. 4	Adult	1st half hr.	0	3	0	0	3
		2nd half hr.	0	3	0	0	3
		3rd half hr.	Apparently slept through this period				
		4th half hr.	0	3	1	0	4
			Total consumed in 2 hr.				10
Aug. 4	Adult	1st half hr.	2	8	1	0	11
		2nd half hr.	2	3	1	0	6
		3rd half hr.	0	3	0	0	3
			Total consumed in 1½ hr.				20

remain long upon the trees. In all probability there is only one generation in Nova Scotia, and the most reasonable assumption is that the adults hibernate in proximity to the orchard.

The larvae spend most of their time in crawling over the leaf surface. Apparently this activity was for the purpose of obtaining food. Whenever contact is made with a host it is seized and the contents sucked out. The larvae usually spend from three to five minutes emptying the contents of an egg or the body of an immature form, and from five to eight minutes in consuming an adult.

The adult beetles crawl quickly over the leaf. Apparently there is no instinct directing them toward their host, and it is simply by chance that they come upon the mites in their irregular course. Even with only a few mites on a leaf, it is remarkable how adept these creatures are at finding and devouring one mite after another. As a rule they consume only the interior of the mites, but sometimes they devour the whole body. The contents of an egg or young mite is usually devoured in about one-half to one minute, and an adult mite in from one to two minutes. When the appetite of the beetle apparently has been satisfied it may remain at rest on the leaves for periods of one-half to one hour. When thus at rest it is entirely in repose as if asleep. If disturbed it usually only crawls to another position and assumes the same attitude, as if resenting the disturbance.

It is apparent that this predator is one capable of destroying large numbers of mites. In a number of orchards, during the summer of 1932, from one to three larvae were present on many of the leaves. In these orchards very material reductions of the pest occurred in a comparatively short time. Should *Stethorus punctum* Leconte become somewhat more numerous and more widespread, it would probably rank next to *Seiulus pomi* Parrott in value as a predator. Notwithstanding lack of lumbers, it must still be considered as one of the more important predators attacking European red mites in the Annapolis valley.

No apparent ill effects to the adults or larvae were noted when spraying with nicotine solution. The minute hairs which clothe the body seem to prevent the insect from becoming wet with the spray.

Plagiognathus obscurus Uhl.

Late in July and early in August, 1932, the adults of *Plagiognathus obscurus* Uhl. were observed in a number of orchards, particularly at Lakeville, N.S. There were no nymphs present at this time and, owing to the fact that none were observed on any previous occasion, it seems that the adults may have flown in from some other host plant.

The adults particularly frequented the terminal growth where green apple aphids were present, from 10 to 12 being present on some terminals. In a few instances they were observed to pierce an aphid and wholly or partially suck out the contents of the body. On different occasions they were also observed feeding on all stages of the European red mite in a like manner.

Mr. G. S. Walley, Entomological Branch, Ottawa, who identified the species, states that members of the genus *Plagiognathus* are phytophagous and that the above species is usually taken in flower clusters, commonly on Compositae. This would lead further to the belief that *Plagiognathus obscurus* Uhl. does not breed on apple and that possibly this predaceous habit is somewhat unusual.

The adults were very shy and at the least disturbance dropped from the tree, but took wing before reaching the ground, and would alight upon another branch. On account of their elusive habit it was difficult to observe their feeding for any extended period. However, an adult which was observed for a ten-minute period consumed two eggs, three immature forms, and one male adult of the European red mite. It would appear from what little is known of *Plagiognathus obscurus* Uhl. that possibly its predaceous habits may not be very consistent. However, in the few orchards where they were most numerous large numbers of mites were consumed.

Camptobrochis nebulosus Uhler

A few nymphs of this species were observed feeding on European red mites in an orchard near Bridgetown, N.S. The nymphs were reared and the adults identified by Mr. G. S. Walley. This species is a member of the subfamily Deraecorinae to which belong a number of species of predatory miridae. The nymphs were first observed on July 29 and at this date were all in the last instar. Adults began to appear on August 2, but nothing further is known of the life history of this insect. The nymphs were so near maturity that there was little opportunity to observe their predaceous habits. However, on a few occasions they were observed for ten minute periods. During these short observations each nymph consumed on the average one egg, one adult and four immature forms. This predator was not found in sufficient numbers in any orchard during 1932 to have any appreciable effect upon the mites. The writer recalls observing the nymphs of this species at Wolfville, N.S., in 1924 and 1925, and as nearly as can be remembered, they were considerably more numerous at that time than during the summer of 1932.

The predators which have been discussed above are apparently the most important that attack the European red mite in Nova Scotia orchards. However, several other predators, the value of which it was difficult to estimate, were observed feeding on mites from time to time. Had these been observed in greater numbers more data could have been obtained relative to their predaceous habits. The following are the minor predators:—

Anystis agilis Banks

This is one of the larger mites and is bright red in color. It runs rapidly over the foliage in a haphazard manner, evidently in search of food. Banks mentions that it feeds upon aphids, small caterpillars and young larvae of the currant sawfly. The writer has noticed it feeding on the European red mite on several occasions. Apparently whenever *Anystis agilis* Banks in its rapid movement over the foliage chanced upon the various forms of *Paratetranychus pilosus* C. and F., it seized and devoured them. In the vicinity of Annapolis Royal, *Anystis agilis* Banks was common. In fact it was thought sufficiently numerous to have an appreciable effect in reducing the European red mite. It was in late June and early July when most were observed. In the main fruit belt where spraying was general this predator was observed only on rare occasions.

Thrips

On several occasions an unidentified species of thrips was observed to attack young and adult mites. The body contents of the mites were sucked out in some manner. These thrips were observed during late August and early September and all at that time were in the immature stages. It was evident that this species of thrips is a more or less general predator for it was observed to seize and suck the body fluids of a leaf hopper nymph as well as the mite *Seiulus pomi* Parrott. During 1931 this thrips was seen frequently, and in a few instances was fairly abundant, but in 1932 very few individuals were observed. It is only in the event of this predator becoming sufficiently numerous that it possibly could be rated as an important natural control factor in relation to the European red mite.

Adults of this thrips have not been observed. A few immature forms were forwarded to the Entomological Branch, Ottawa, for identification, but these proved insufficient. It was stated, however, that they probably belonged to the genus *Haplothrips*. Viewed from above, the color marking of the fully grown larva of this thrips was as follows:—Prothorax red on lateral margins, darker on dorsum; mesothorax yellowish; metathorax red; Abdominal Segments 1 and 2 yellowish with some fine dark pepper markings; 3 and 4 red; 5 and 6 yellowish; 7 and 8 red with a few dark markings; taking Segments 9 and 10 together, the first third black, followed by a narrow transverse band of red with the cauda black.

Syrphid Larvae

On a few occasions syrphid fly larvae were observed to seize a quiescent European red mite and consume the larger portion of the body. It is not likely that these larvae could capture adults or active young mites, and it is not known whether they would favor mite eggs. Under the most favorable circumstances it is not likely that this group of predators will become important enemies of the European red mite. It is necessary that the larvae have near at hand an abundance of food such as aphid clusters, among which they are frequently found. The feeding observed upon mites was probably more or less a chance occurrence.

Campylomma verbasci Meyer

This is a small capsid bug, the adults of which were observed to be very numerous in a few orchards during 1931. The adults of this predator were observed attacking only the adult mites, but eggs may also be consumed. These bugs were the most numerous on July 9, and only a few days later they had largely disappeared. This insect has two generations in Nova Scotia. The first brood adults migrate to the potato plant where they feed for some time before returning to the apple.

The adult bugs do not search in any systematic way for mites as other capsids do, but seize them as they chance upon them in their erratic movement over the foliage. They frequent the terminal growth of the apple, especially trees infested with aphids. They were not observed feeding upon aphids but it seems likely that this occurs. One adult stung the back of the writer's hand sharply.

While these bugs were present large numbers of mites were consumed. This was very evident in one orchard. However, although large reductions of mites may occur on some occasions, this is not considered a predator of any great consequence. Its appearance in numbers is too infrequent and its stay is apparently very short. During 1932 this insect was observed only on a few occasions and then in small numbers.

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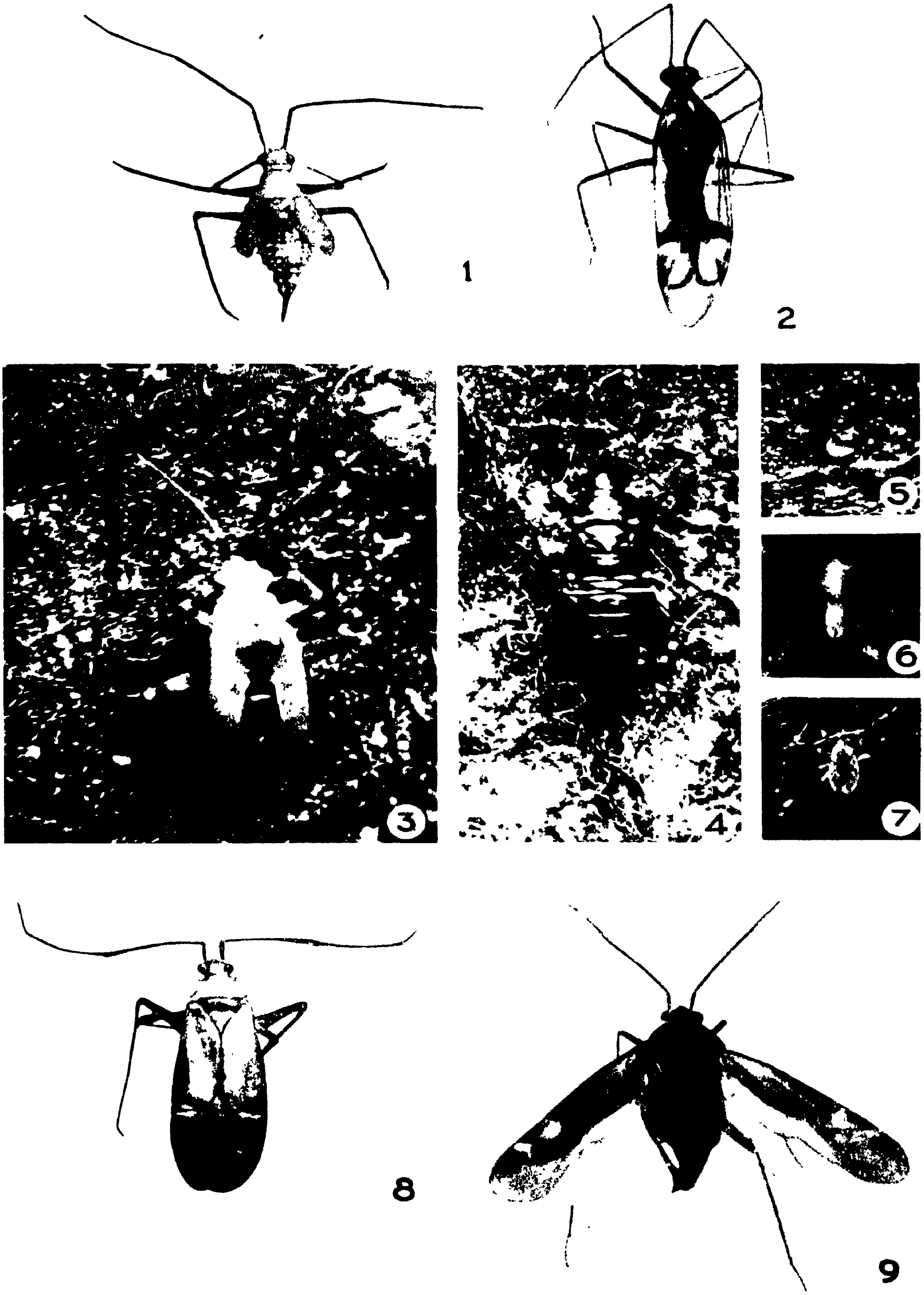

1, Last stage nymph of Hyaliodes vitripennis Say; 2, H. vitripennis Say, adult; 3, Last stage nymph of Diaphnidia pellucida Uhl.; 4, Unknown predator on European red mite, probably Nabid; 5, Egg of Seiulus pomi Parrott; 6, Egg of Campylomma verbasci Mey.; 7, Seiulus pomi Parrott, adult; 8, Diaphnidia pellucida Uhl., adult; 9, Plagiognathus obscurus Uhl., adult.

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LIGHT AS A CONTROLLING FACTOR IN THE GROWTH OF *BALANUS BALANOIDES*[1]

BY A. BROOKER KLUGH[2]* AND CURTIS L. NEWCOMBE[3]

Abstract

Areas on different sides of the wharf of the Atlantic Biological Station that had been denuded in the spring and that had different degrees of illumination were selected and the growth of *Balanus* followed throughout the summer season. The results show (i) that there is a significant correspondence between rate of growth of *Balanus* and the amount of illumination, (ii) that the difference between the amount of growth at widely separated vertical levels is quite similar to the difference in the increment of growth in areas on the same level which are subject to wide variations in the amount of illumination, and (iii) that during the summer of 1931, there was no appreciable set of barnacles after June 6 in the St. Andrews region. The presence of a large number of elongated barnacles may result from the operation of one main factor or from the combination of several. It may indicate (i) that there has been a very heavy set, in which case exceptionally favorable growing conditions cannot necessarily be inferred, or (ii) a rapid rate of growth even though the set has not been particularly thick, or (iii) the presence of favorable growing conditions together with a good set. Each of these possibilities should be considered in the evaluation of *Balanus balanoides* as an organic indicator.

Introduction

During the course of ecological work on intertidal animals, it was observed that barnacles, *Balanus balanoides*, growing on the wharf at the Atlantic Biological Station, St. Andrews, New Brunswick, showed size variations in areas exposed to different light conditions.

Observations made during the summers of 1929 and 1930 suggested the probable importance of light as a controlling factor of growth and resulted in the formulation of our hypothesis, namely, that light intensity plays a far greater role in the growth of the invertebrate fauna of the intertidal belt than has heretofore been realized. Measurements were made on August 22, 1929, of a *Balanus* community growing on the under side of a ledge of sandstone conglomerate occupying a position in the intertidal belt near the mean low water level. The barnacles were packed closely together and their average length and width were practically the same, namely, 18 mm. In no other part of the intertidal zone were specimens found that had attained these dimensions. This, we believe, was due to the fact that growth is favored not only by the low level but also by total protection from direct sunlight (2). Again, on a movable beam which supports the float on the south side of the wharf, barnacles of the same set were found to be about twice as large on the "north" side of the beam (*i.e.*, on the inside surface very near the wharf) as on the exposed "south" side. Consequently, during the summer of 1931, areas A_1, A_2 and A_3, on the outside of the wharf at the same tidal level but with different degrees of illumination, were studied and also areas at different tidal levels for comparisons with the above. (Fig. 1).

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[1] *Manuscript received June 10, 1935.*
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**The sudden death of Professor Klugh has made it necessary for the final revision of this paper to be made by the junior author.*

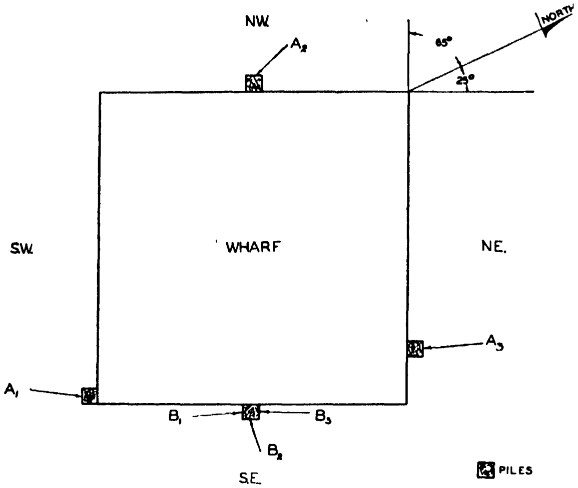

FIG. 1. *Diagram showing the approximate position of the several quadrats selected for study on the wharf at the Atlantic Biological Station, St. Andrews, New Brunswick.*

On the three exposed sides (B_1, B_2 and B_3) of a vertical wharf pile, 18 cm. in cross section extending out from the centre of the "east" side of the wharf, areas which were covered with barnacles of this year's growth were selected for intensive study. The position of the wharf is such that the side hereafter termed the west side (A_2) faces 65° west of north—true bearing—(Fig. 1). The quadrats A_1, A_2, A_3, and B_1, B_2, B_3 are listed in decreasing order of magnitude with respect to the total amount of illumination.

Methods

The light measurements were made with a Bell and Howell photometer, Model B, an instrument designed for photographic purposes. The principle of this instrument is that the image of the filament of a small bulb is matched in intensity against an object which is viewed through the photometer, the image being superimposed upon the object. The current is supplied by a flashlight battery in the handle of the instrument and is controlled by a rheostat, the turning of which is indicated on a scale on the barrel. For details concerning this instrument including its efficiency and methods of use for ecological purposes, the reader is referred to a paper by the senior author (1). The manner in which the instrument was used in the present investigation was to place a neutral gray card in front of, and parallel to, the quadrats, and to reduce the readings made, with this card as a standard surface, to percentages of the highest intensity recorded.

Counts and measurements of barnacles were made on June 6 and 13, July 14, Aug. 4–6, and Sept. 8. Records of abundance were obtained by counting the barnacles on several centimetre and decimetre square areas chosen at random, and then computing the arithmetic averages. The maximum height and width were recorded and counts were made of the numbers in each size group. On a basis of these results, it has been possible to determine the variation in amount of growth on the different sides of the wharf and also the increments of growth during certain periods throughout the season.

An expression of the variation in growth and numbers of barnacles in the quadrats studied does not lend itself readily to statistical treatment. This will be apparent to the reader in view of the widely felt lack of satisfactory quantitative methods for community study (3). Therefore, it has been considered impractical to attempt an accurate mathematical representation of the increments of growth. This, however, does not prevent a fairly accurate estimate based on numerous observations of the selected quadrats and on considerable cruising in the barnacle communities of the adjacent intertidal area. The fact that there are such significant differences in the growth increments at the various stations minimizes the need for a more refined treatment.

Results

Our observations clearly demonstrate a correspondence between barnacle growth and amount of illumination. In view of the fact that all the stations were within 14 metres of each other, it is reasonable to assume that other determining factors are quite similar. From previous observations it had been noted that the dogwhelk *Purpura lapillus* preyed upon *Balanus balanoides*, hence in order to insure that these predators would not invade the habitats under observation, screens of wire netting were erected to exclude them.

Measurements and counts made near quadrats A_1, A_2 and A_3 definitely indicate an increase in the rate of growth of the different classes accompanying a decrease in amount of illumination.

On June 6, 1931, an examination was made of barnacles, two years old, growing on areas immediately adjacent to each of the three quadrats. They were found to be comparatively uniform in size and distribution. Those adjacent to A_1 were approximately 5 mm. in height and 6 mm. in width; those adjacent to A_2, 7 mm. in height and width; and those near A_3, 15 mm. in height and 9 mm. in width.

The stations that were denuded on October 30, 1930, were observed on February 31, 1931, and a practical absence of plants and animals was noted. The next records were made during the second week in June when, on the basis of the different sizes of barnacles present, it was concluded that there had been, up to that time, at least two distinct sets at intervals of about two weeks. To determine the extent of any subsequent set that might take place, an area 9.5 cm. by 15 cm., near quadrat A_1, was denuded together with an area 19 cm. by 20 cm., adjacent to quadrat A_3.

Examination of these areas on July 14, 1931, gave the results shown in Table I.

TABLE I

Date	Quadrat	Number	Species	Height	Width
July 14, 1931	Near A_1	3	*B. balanoides*	1–1.5 mm.	1–1.5 mm.
		7	*B. balanoides*	< 1 mm.	< 1 mm.
	Near A_3	13	*B. balanoides*	1–1.5 mm.	1–1.5 mm.

It appears, therefore, that during this season the set of barnacles after June 6 was of minor importance.

Light Data

It is obvious that the data on the light factor most desirable for the purpose of this investigation would be the total amount of light received by each quadrat during the course of the investigation. It is equally apparent that such data are extremely difficult to obtain because of the large number of variables involved; the weather conditions varying from day to day and from hour to hour, the intensity of the sunlight decreasing from June to September (the period covered by these observations), and the tidal amplitude varying from day to day so that the quantity and the quality of the light reaching a quadrat while under water varies from day to day. The data which are of significance in giving a picture of the light conditions in this region are as follows: The pyrheliometer value of full noon sunlight at this station varies from 1.55 gm. cal./cm.^{2}/min. in September. The sunlight first strikes the "south" side of the wharf (A_1) at 8 o'clock (sun time) and leaves it at 8 p.m., or at sunset. It is on the "west" side (A_2) from 2 p.m. until sunset while it is only in June and early July that the early morning sun is on the "north" side (A_3) for about an hour. Of the quadrats on the vertical pile beneath the plank walk of the wharf the only one which receives any appreciable amount of sunlight is B_1 and it is exposed to the sunlight during only a short period in the morning. The light values of the quadrats for different weather conditions and times of day, as read on the Bell and Howell photometer and expressed as percentages of the highest value obtained, are shown in Table II.

TABLE II

——		A_1	A_2	A_3	B_1	B_2	B_3
Bright sun	9:30 a.m.	5	5	13	2	4	2
Bright sun	2:00 p.m.	100	90	7	17	8	3
Cloudy-bright	4:00 p.m.	25	50	12	13	7	8
Foggy	9:00 a.m.	13	17	30	2	4	2

The significant point of difference with respect to the light conditions in the respective quadrats—A_1, A_2, A_3 and B_1, B_2, B_3, lies not so much in the variations in actual intensity during one particular time of day as in the variations in total amount of illumination resulting from differences in the length of the period of exposure to sunlight.

Barnacle Data

During the second week in June, measurements and counts were made at quadrats A_1, A_2, and A_3, the data obtained being shown in Table III.

TABLE III

Date, 1931	Quadrat	No. of specimens per dm.^{2}	Height, mm.	Width, mm.
June 13	A_1	300	2–4	2–4
		2060	<1 mm.	<1 mm.
June 6	A_2	1100	4–5	4–5
		2000	1–2	1–2
June 13	A_3	1200	5–8	4–6
		800	1–5	1–3
Aug. 4	A_1	220	4–7	4–7
		210	3–4	3–4
		270	2–3	2–3
		370	1–2	1–2
		250	<1 mm.	<1 mm.
Aug. 6	A_2	600	7–8	4–5
		200	3–4	2–3
		100	2–3	1–2
Aug. 4	A_3	600	8–13	4–7
		700	5–6	2–3

From Table III, it is evident that there are significant differences in the amount of growth and in the numbers of individuals within the size groups. Also, it is clear that the amount of growth is in inverse proportion to the amount of illumination incident upon the habitats. On the basis of the data collected in August, 1931, Fig. 2 has been constructed. For comparative purposes, the approximate volume has been calculated by multiplying the maximum width squared, by the height and by the number of specimens. Comparing the two sets of data presented above and considering in each case the numbers of the largest and smallest classes, it is seen that there are far greater numbers of large barnacles in quadrat A_3, less in quadrat A_2 and still less in quadrat A_1, and that the reverse is true in the case of the small barnacles. We consider this to be due chiefly to two factors:

(i) Increase in amount of growth which, as is particularly evident in the case of those less than 1 mm. in height, results in a marked reduction in the numbers of this class at quadrats A_2 and A_3.

(ii) The lethal effect produced by the mechanical action of crowding under conditions favorable for growth accounts for an almost total absence of barnacles less than 1 mm. in height in quadrats A_2 and A_3 (2).

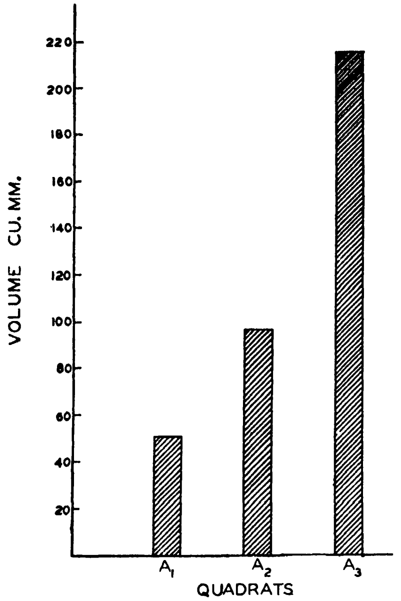

FIG. 2. *Effect of variations in the amount of illumination between quadrats A_1, A_2 and A_3 on the growth of Balanus balanoides. Width^2 × height × number of specimens is approximately equivalent to the volume which is expressed in thousands of cubic millimetres per decimeter square.*

The efficacy of the last-mentioned factor in accounting for reduction in numbers of small barnacles is suggested from observations at quadrat B_3 where there have been observed numerous dead specimens less than 2 mm. in height.

The difference in the amount of growth in quadrats A_1 and A_2 is probably less than that in quadrats A_2 and A_3, while the difference in the amount of growth in quadrats A_1 and A_3 is very definite.

The amount of growth in quadrats B_1, B_2 and B_3 already described is also proportional to the difference in the amount of illumination, thus being in agreement with results obtained from quadrats A_1, A_2, and A_3.

It is not unusual to find areas in which the barnacles are crowded and unusually long. A not uncommon interpretation of this phenomenon is that the crowded condition is a result of thickness of set. It was observed that the set on the different sides of the wharf was approximately equal. In quadrat B_1 and also quadrat A_1, there was no evidence of crowding on August 4. Some crowding was noted at quadrat B_2 and considerably more at B_3. In the light of these findings it appears that increased elongation may be interpreted as due to crowding which, in turn, is due to favorable growing conditions and not necessarily to an unusually thick set (See 4 and 5).

Acknowledgments

The authors take pleasure in acknowledging the aid received in the form of facilities from the Biological Board of Canada and in the form of instruments from the National Research Council of Canada.

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THE MOLLUSCAN FAUNA OF MEACH LAKE, QUEBEC[1]

BY A. LA ROCQUE[2]

Abstract

The shallow water zone (0–10 ft.) of Meach Lake provides three types of bottom:— rock, sand and mud. Certain species of mollusca live in all three habitats, some in two and some in one. Seventeen species were found, two of which were confined to the drainage. Nine of the seventeen are recorded for Meach Lake for the first time. The number of species and the size of individuals are both smaller in the inlet and in the streams flowing into the lake than in the lake itself. A dwarfed fauna occurs at the outlet. One species has apparently disappeared from the lake in a period of two years.

Introduction

The Gatineau valley is the favorite resort of thousands of tourists and fishermen to whom its lakes are a major attraction. Without the lakes the region would lose its appeal; hence it is most important to learn as much as possible of the complicated associations of life forms which help to maintain conditions as they are in these lakes. The role of the mollusca in the maintenance of this equilibrium is one of major importance, a fact appreciated by all biologists who have to deal with lake life. This paper deals in detail with the mollusca of one lake, their ecology and distribution. When more data of a similar nature are available for other lakes it will be possible to estimate the relative value of the mollusca in them as fish food, purifying agents, scavengers, etc., thus providing a better understanding of the means of preserving our lakes in their present state.

This paper embodies the results of observations made on various weekend trips to Meach Lake, and one week devoted to collecting material for the National Museum of Canada in September, 1934.

Meach Lake was chosen as one of four lakes of different types in eastern Canada whose molluscan fauna is to be compared in an exhibit in the National Museum. This exhibit will show the differences brought about by various environmental conditions and attempt an explanation of their causes; the other lakes chosen are MacKay Lake, near Ottawa, White Lake in Renfrew County and Lake Erie.

The peculiar character of the mollusca of Meach Lake has long been known to Ottawa conchologists. As long ago as 1880, Heron (3) recorded a peculiar *Physa* which he identified as *Physa lordi* Baird and which was later distinguished from it by Baker under the name *latchfordi*. Latchford and Poirier (12, p. 132) called attention to the large size of the *Helisoma anceps* found there. Since then the lake has been visited many times by conchologists, and their published results are discussed in a section of this paper.

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[1] *Manuscript received May 30, 1935.*
Contribution from the Bureau of Economic Geology, Department of Mines, Ottawa, Canada. Published by permission of the Director.

[2] *Museum Assistant, Division of Palaeontology, Geological Survey.*

The writer visited Meach Lake on three occasions: June, 1932, August, 1933, and September, 1934. These visits produced many specimens of species already recorded and ten species not previously recorded for the lake. All the material collected was deposited in the National Museum of Canada.

Methods

The primary object of the work being to obtain specimens for the Museum, very little time could be spent on the examination of the deeper parts of the lake. It was known from earlier visits that the lake could be divided into three habitats, more or less grading into each other and an exact knowledge of the relative proportions of these habitats was obtained by rowing slowly

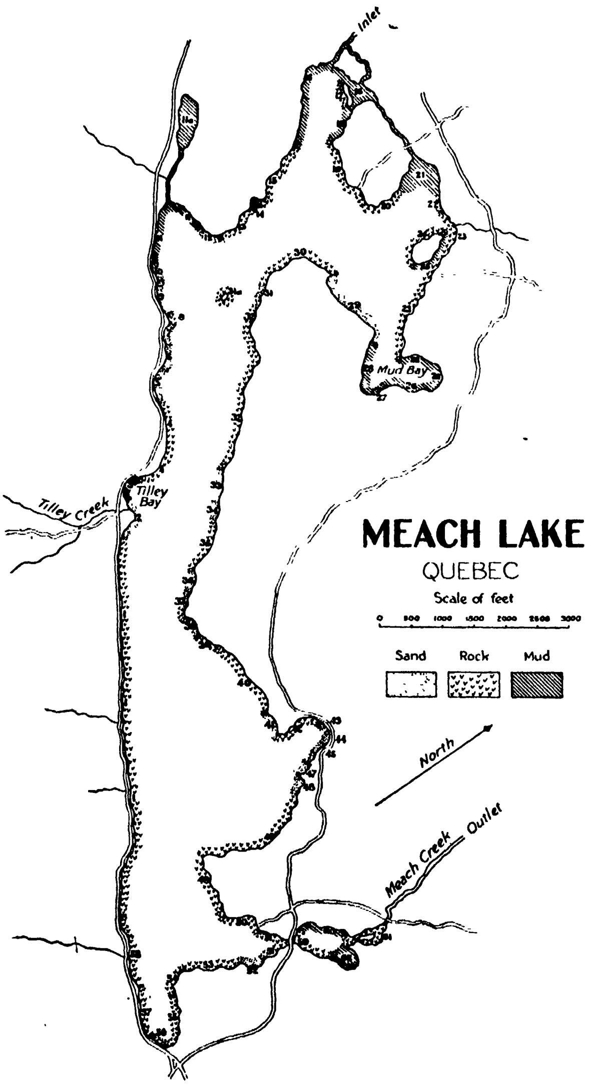

FIG. 1.

around the lake, stopping wherever a change occurred to collect specimens. The character of the bottom and the species present were then carefully recorded, the extent of the station being indicated on the map. Collections from each station were kept separate so that the specimens from each could be compared later. After a while it became evident that the same species could be found on a given type of bottom and specimens were noted only, not collected.

No deep water dredging was done, but species could be recognized in water as deep as ten feet, the water of the lake being reasonably clear. Since it has been the experience of most collectors to find mollusca most abundant within the ten-foot contour, it is probable that dredging would not materially increase the number of species found except perhaps for *Pisidium* and *Valvata.*

All collecting was done by hand, and the majority of specimens collected were alive. A hand sieve and rake* were also used, the former being very useful in obtaining *Sphaeriidae* and the latter for *Campeloma.*

As the writer has not seen the rake method described in the literature, and can vouch for its effectiveness, a brief description of it may be useful: Use an ordinary garden rake with a long handle. Rake the sand over a given area, say 4 × 4 ft., lightly or deeply according to the species sought. Wait until the sand settles again and the water clears. The specimens dislodged by the rake are now in plain sight and can be picked up by hand. This method produced *Campeloma* by the dozen where only a few specimens had been obtained by hand or with the hand sieve. The tell-tale mound which indicates the presence of the species on a clay bottom was not observed but dead shells along the shore and on the sand indicated the presence of the species in the vicinity.

Physiography

The correct interpretation of the peculiar fauna of Meach Lake must rest on an understanding of its situation. The lake is the fourth in order of the four major lakes of the Meach Creek drainage which are: Taylor, Philip, Harrington and Meach. Most of its water comes from Harrington Lake, through a winding, shallow inlet, and a small amount is also derived from the half-dozen creeks which drain into it. The outlet is through McGoey lake into Meach Creek, thence into the Gatineau River.

The lake is long and narrow, with a few shallow bays. Where the shore is rocky the hills descend abruptly into the lake, the water deepening quickly.

The northwest wind, which is not uncommon, has a tremendous sweep along the entire length of the lake and raises fair-sized waves which dash against the rocky shores. The exposed portions are accordingly unfavorable to mollusca, and at the southeast end, where the cliffs are almost vertical, none are found.

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* *The rake method was shown to the writer by Mr. C. H. Young of the Museum staff, who has used it to advantage in collecting both marine and fresh-water shells from sand.*

The outlet of the lake has been dammed so that the water level is fairly constant. Aside from this dam, human influence on the lake is slight. Along the southwest shore, especially near the east end, quantities of boulders were dumped into the lake to widen the road-bed, changing a sand-clay habitat to rock. Sewage dumped into the lake is negligible. A sawmill which ceased operations many years ago may have caused an accumulation of sawdust in the past but no sign of it is to be found at present.

Previous Work

The first record of shells from Meach Lake is by Heron (3) 1880. Four species are enumerated: *Unio complanatus* (Dillwyn), *Planorbis bicarinatus* Say, *Limnaea stagnalis* Linn. and *Physa lordi* Baird.

The next paper is by Latchford (6), 1882. It mentions three species from Meach Creek, one from McGoey Lake and two from Meach Lake, only one of which, *Anodonta marginata* Say, had not been recorded by Heron.

Two species were added to the list by Small and Symes (15), 1882: *Limnaea megasoma* Say and *Limnaea lepida* Gould. The latter was shown by Baker (1, p. 140), to be a misidentification.

The next two papers to mention the lake (12, 14) merely confirm the finds already made and add no new species to the list.

In another paper by Latchford and Poirier (13) two *Sphaeriids* are added to the list, the first from McGoey Lake and the other from Meach: *Sphaerium simile* (Say) and *Musculium rosaceum* Prime. The seven other papers in the reference list that deal with the lake, add no new species to the list.

The writer's 1932 collection added five more species to the list: *Helisoma campanulatum wisconsinense* (Winslow), *Helisoma trivolvis pilsbryi* (F.C.B.), *Campeloma* cf *decisum* (Say), *Pseudosuccinea columella* (Say), and *Succinea retusa* (Lea). The 1934 collections added five more: *Pisidium* sp., *Gyraulus parvus* (Say), *Physa gyrina* Say, *Ferrissia parallela* (Say) and *Amnicola ?limosa porata* (Say). This brings the number of species to seventeen, as follows:—

Musculium rosaceum Prime
Pisidium sp.
Anodonta marginata Say
Elliptio complanatus (Dillwyn)
Lymnaea stagnalis lillianae F. C. Baker
Bulimnaea megasoma (Say)
Pseudosuccinea columella (Say)
Gyraulus parvus (Say)
Helisoma trivolvis pilsbryi (F. C. Baker)
Helisoma campanulatum wisconsinense (Winslow)
Helisoma anceps latchfordi (Pilsbry)
Physa parkeri latchfordi F. C. Baker
Physa gyrina Say
Ferrissia parallela (Say)
Amnicola ?limosa porata (Say)
Campeloma cf *decisum* (Say)
Succinea retusa (Lea)

Discussion of the List

This assemblage of species shows a typically northern molluscan fauna, very much like that of other Gatineau Valley lakes in the abundance of species like *Lymnaea stagnalis*, *Campeloma decisum*, *Ferrissia parallela*, *Pseudosuccinea columella*, etc. With these other lakes it shares a scarcity of representatives of the genus *Stagnicola*, the only one to produce these in abunddance being Chilcott Lake, a few miles north of Meach, where *S. laurentiana* Latchford is as common as *Lymnaea stagnalis* in other lakes. Especially notable is the absence of the *S. emarginata* group of which Baker (2, p. 234) says: "The habitat is normally in lakes or large rivers, often in situations where there is violent wave action." The species is present in the Rideau River; the lake presents the right kind of habitat, yet no *Stagnicola* is to be found there.

But Meach Lake resembles other Gatineau Valley lakes in another respect, the tendency to produce unusual variations. So far as is known the nearest locality for *Helisoma trivolvis pilsbryi* is Oneida Lake, in New York State; the nearest *recorded* locality for *H. anceps latchfordi* is Brome Lake, Quebec, but these may turn out to be the nearly related variety *portagense* described from Maine. *Physa parkeri latchfordi* has not been recorded for any locality between Michigan and Meach Lake.

Another remarkable feature is the absence of Naiades of the genera *Lampsilis* and *Alasmidonta* which are common, the one in Gauvreau, Grant and Blue Sea Lakes and the other in Taylor and Bernard Lakes.

Systematic Account of the Species

The ecological data which are summarized in the tables are assembled here under each species and discussed in detail. Certain varieties have been differentiated which did not appear in previous work and a discussion of the characters which led to this differentiation is given.

Musculium rosaceum Prime: This species is reported by Latchford and Poirier (13). One dead specimen of a *Musculium* was collected on sand mixed with some clay at Station 12 but no live specimens were obtained anywhere in the lake. While it is very probable that this specimen belongs to the species *rosaceum* the group is so difficult that a definite identification is best left to a specialist.

Pisidium sp.: At the same station one specimen of a *Pisidium*, also dead, was obtained. Live specimens, perhaps of the same species, were fairly frequent in the creek emptying into Tilley Bay; dead specimens were found as far as twenty feet out in the bay, but only in the immediate vicinity of the mouth of this creek.

Anodonta marginata Say: Common in mud habitat, group B. In August 1933 gravid females of this species were collected but when examined for glochidia they were found to contain only eggs. The same condition obtained in gravid females collected in September 1934. Baker (2, p. 166) found "none gravid in July and August" and of the breeding season says: "probably similar to that of *Anodonta grandis*", that is, glochidia discharged very early. Since the glochidia were not mature in September it is unlikely that they are discharged (in *A. marginata*) until the Spring.

Elliptio complanatus (Dillwyn): This species is rare in the rock habitat, commoner in sand and entirely absent from the mud. The specimens are very compressed and of small size. Umbones were eroded in all specimens examined. No glochidia observed.

Lymnaea stagnalis lillianae F. C. Baker: Found in all habitats; thrives on sand and rock, but rare on mud. Material from Meach Lake was included in a supplement to the Ottawa list published recently by the writer (5, p. 34). The aperture-spire ratio is fairly constant, the aperture being longer than the spire in all but two specimens, both of which were from mud in protected bays. The dwarfed specimens are discussed below in the section on the dwarf fauna.

Bulimnaea megasoma (Say): The only specimens found by the writer came from a rock habitat (Station 17) and were collected in 1933. In 1934 the species could not be found anywhere in the lake. Many of the papers dealing with the mollusca of Meach Lake record *Bulimnaea megasoma*. Small and Symes (15, p. 57) state that the species was first found there "by Mr. Latchford in September 1880, and a good series was secured during the past season (1882)." Poirier mentions it again in the next year (14, p. 74) and with Latchford in 1885 records it for McGoey Lake. So far no specific locality in Meach Lake had been given. In the next two papers in which he mentions the species Latchford gives first "in a bay near Mr. Tilley's cottage, Meach Lake" and then, more specifically: "in a sheltered bay two hundred yards north of the Tilley cottage." Exactly what bay was meant cannot be ascertained definitely but if the distance is stretched a bit Mud Bay may have been meant. Specimens were plentiful since a good series was secured.

In 1934 Mud Bay was carefully searched, but no specimens were to be found. The same applies to Station 17 where specimens were obtained in 1933. It seems that *B. megasoma* has been losing ground in Meach Lake until now it is almost certainly extinct. A particularly careful search was made of all likely stations in the lake itself, in the inlet and in McGoey Lake, without success.

The causes of this extinction can only be guessed at, but the following may have had some effect: (a) raising of the level of the lake by the dam at the outlet; (b) the unusually severe winter of 1933 which came on very suddenly at the end of October and lingered into April; (c) pollution of the lake by sewage. The last is extremely unlikely since very little sewage is emptied into the lake and the water always appeared clear and clean.

Pseudosuccinea columella (Say): Abundant on water lily leaves, mud habitat. The specimens collected were small as a general rule, the largest being 16 mm. in length.

Gyraulus parvus (Say): Rare on water lily leaves, mud habitat. The species was determined by Mr. F. C. Baker.

Helisoma trivolvis pilsbryi F. C. Baker: Common on water lily leaves in the mud habitat, rare in the rock habitat, none found on sand. Adult specimens are consistently larger than the variety found in the Ottawa River, which is not quite typical according to Mr. Baker. In all the specimens collected the inferior carina is absent, the nuclear whorls regularly rounded below and with only very minute revolving lines. This character alone would distinguish the specimens from typical *trivolvis* and *infracarinatum*, both of which have a well-marked carina on the umbilical side of the nuclear whorls in the majority of cases, rare specimens having faint revolving lines. This record of *pilsbryi* is the first for the Ottawa district. The specimens were identified by Mr. F. C. Baker. It is probably present in Taylor and Philip lakes but so far no specimens have been collected in either lake.

Helisoma campanulatum wisconsinense (Winslow): Common on water lily leaves, mud habitat, and frequent on sand and rock. This species seems adapted to thrive in all three habitats. The Meach Lake specimens are placed in this variety on the strength of their axial height and slightly carinate whorls. The spire is not as elevated above the last whorl as in specimens from Tomahawk Lake, Oneida County, Wisconsin, nor are the whorls as strongly carinate. But the same variation may be seen in specimens of *wisconsinense* from Blue Sea Lake, Quebec, considered by Miss Winslow to belong to this variety.

Helisoma anceps latchfordi (Pilsbry): It seems that a combination of shallow water and sand bottom is necessary for the well-being of this variety. Occasional specimens were found on rock and mud, but only a few in each case. As may be seen from the list of sand habitats below, whenever the water deepens to more than three feet *latchfordi* disappears, to reappear again when it becomes shallower.

Lake specimens of the variety are fairly constant in height, width of umbilicus, strong carination and shape of aperture. The spiral lines, which are very faint on the superior and inferior walls of the shell, tend to disappear entirely on the side of the whorl between the carinae. In any case, the spiral lines, when present, are so faint as to be indistinguishable except under a strong magnification. They appear as fine wrinkles between the vertical riblets, upon which they do not impinge. The importance of this character will appear when the fauna of Taylor Lake is discussed; the *latchfordi* from this lake have strong spiral lines which cut the riblets and are visible without the aid of a lens.

Physa parkeri latchfordi F. C. Baker: This variety thrives on both sand and rock. The assertion made by the writer that it was absent from the gravelly parts of the lake (4, p. 134) was due to insufficient collecting*. In the mud habitat this species is replaced by *P. gyrina* Say.

**In addition a printer's error made the passage read: "Both snails are absent from exposed gravelly parts of the lake which is covered with cat-tails." In the original this read: "Both snails are absent from exposed gravelly parts of the lake and from the bay at the west end which is covered with cat-tails."*

Specimens from Meach Lake sent to Dr. W. J. Clench were returned by him marked *Physa parkeri* Currier. While the two species are rather closely allied, still there are differences which enable one to separate them. In *latchfordi* the shell is thinner than in *parkeri*, the spire less flattened and the columella less twisted. Adult specimens of *parkeri* from the type locality are also much larger than any specimen from Meach Lake yet seen. These differences, while they are admittedly slight, are still sufficient to separate groups of specimens from the two type localities, and to preclude the dropping of *latchfordi* and its inclusion under *parkeri* as a direct synonym.

The Meach Lake form must be treated in one of two ways, either as an independent species or as a variety of *parkeri*.

Its treatment as a variety of *parkeri* would imply a derivation from that species and the presence at some time in the past of the species in localities intermediate between Michigan and Meach Lake. So far no such intermediates are known.

Its treatment as a species would imply derivation from the same stock, possibly now extinct, and parallel development in response to lake environment. It would not imply the presence of the species in intermediate localities.

The first alternative could be considered more judiciously if we knew more about the *Physae* of the intervening territory. So far *P. parkeri* has not been recorded anywhere in Ontario or New York but there are many lakes, at least in Ontario, where no collecting has ever been done. On the other hand the areas studied, such as the Toronto, Hamilton and Ottawa regions and the lakes of extreme western Ontario, have not yielded any specimens of *parkeri*. The species is so remarkable, both in size and appearance that any collector would rate it an extraordinary find and would not fail to record it in a local list.

The second alternative requires a greater knowledge of the genitalia and radula of the two forms and of the origin of both. But in either case the differences in the shell alone appear sufficient to retain the name *latchfordi*. In deference to Dr. Clench's greater experience the form is here treated as a variety or race of *parkeri* but the writer fails to see how it could be derived from that species. The hypothesis of parallel development from the same stock appears more probable and more in accord with the apparent absence of the species in intervening localities.

Physa gyrina Say: Found sparingly on water lily leaves, mud habitat. Specimens identified by Dr. Clench.

Ferrissia parallela (Say): Fairly common on water lily leaves, mud habitat. The shell is almost invisible on a wet leaf and must be searched for carefully if any quantity is to be obtained.

Amnicola ?limosa porata (Say): Fairly common on water lily leaves, mud habitat. Most of the specimens taken were immature but the globose form, slightly flattened spire whorls and widely open umbilicus place these specimens almost certainly in *limosa porata*.

Campeloma cf. *decisum* (Say): This species is found in sand only. The shells are thin as in the *decisum-milesii* group, resembling the former more than the latter. As a large series of specimens both dry and in alcohol were collected, a study of the characters used by Baker (2, p. 57) in differentiating the species could be made. All the specimens examined were females, most of them with embryonic young, others with eggs only.

The young show a spire with sunken apical whorls as figured by Baker (2, p. 77 and Fig. 33) for *decisum*. There is considerable variation in the erosion of the spire in the adult, specimens varying all the way from those having the spire intact to some in which all but the last two whorls are worn off and which have in addition other worn spots unconnected with the spiral erosion.

Three radulae of this Campeloma were mounted and measured. The central tooth is 30μ wide as compared with 50μ for *decisum* and 40μ for *milesii* as given by Baker (2, p. 57). On this evidence, then, this form approaches *decisum* in the character of the embryonic whorls but is nearer *milesii* in the size of the central tooth.

The specimens are therefore placed doubtfully in *decisum* but it is the writer's belief that they will eventually be recognized as a distinct variety or species. However, since the classification of the group is far from satisfactory, the naming of this form must be delayed until more is known about the species of *Campeloma* already described.

Succinea retusa (Lea): Rare, on water lily leaves, mud habitat. Specimens of both this species and *P. columella* were dissected to make sure of the identification. Both species are present in the same habitat, sometimes one of each on the same leaf but only in one station (No. 26) was *S. retusa* found.

Detailed Description of the Habitats

That part of the shore of Meach Lake which is included within the ten-foot contour below water level may be divided into three main groups according to the character of the bottom. On the whole these groups are fairly distinct but may grade into each other. They may be defined as follows:

1. *Rock:* Varying from sheer rock wall or precipitous rocky shores to boulders interspersed with coarse gravel. The rocks are covered with green filamentous algae in a mat one-eighth to three-quarters of an inch thick. The water usually deepens quickly and vegetation is scarce.

2. *Sand:* Fine sand, with flakes of mica and in spots a little clay. The water usually deepens slowly. This type of bottom is found mostly in small protected bays and is most extensive at the southeast end of the lake.

3. *Mud:* Sticky, soft blackish-brown mud in a layer at least four feet deep in places, supporting an abundant and varied assemblage of water plants.

Each habitat is treated in more detail below.

Fauna of the Rock Habitat

Twenty-four stations of this type were studied, only two of which had no molluscan fauna. The species present are indicated in Table I. From this table it will be seen that the characteristic species of this habitat are *Lymnaea stagnalis lillianae* and *Physa parkeri latchfordi* with *Helisoma campanulatum wisconsinense* a poor third while the other species are represented by occasional specimens only.

Stations 53 and 54, where the rock rises perpendicularly out of the water and is exposed to the full force of the waves, are entirely devoid of shells.

In Stations 37, 39 and 40 where the rocky bottom was strewn with patches of clay and sand, shells were scarcer than on unmixed sand or rock bottom.

The two occurrences of *H. anceps latchfordi* are negligible as in both cases specimens were very scarce. At Station 8 only one live and two dead specimens were collected and at Station 31 only one specimen was seen.

An anomalous occurrence is that of *B. megasoma* on the rocky shore of the island at Station 17 in 1932. In 1934 every foot of this locality was examined but no *B. megasoma* found.

TABLE I

FAUNA OF THE ROCK HABITAT

Species	Stations											
	1	4	8	13	14	17	23	30	31	32	35	37
L. stagnalis lillianae	c	c	c	c	c	c	c	c	c	c	c	r
Physa parkeri latchfordi	c	c	c	c	c	c	c	c	c	c	c	r
H. c. wisconsinense		r	r	r	c	c		c	c	c		
H. a. latchfordi			r						r			
H. t. pilsbryi						r						
Elliptio complanatus						r						
*Bulimnaea megasoma**						r						

	Stations											
	39	40	42	47	49	50	51	53	54	55	57	58
L. stagnalis lillianae	r	r	c	c	c	c	c			c	c	c
Physa parkeri latchfordi	r	r	c	c	c	c	c			c	c	c
H. c. wisconsinense												
H. a. latchfordi												
H. t. pilsbryi												
Elliptio complanatus												
*Bulimnaea megasoma**												

*1932 only.

Fauna of the Sand Habitat

Eighteen stations of this type were examined, which varied greatly in character. The distribution of species is shown in Table II. *Lymnaea stagnalis lillianae* and *Physa parkeri latchfordi* are abundant, as in the rock habitat. Where the water is shallow (0–3 ft.) *Helisoma anceps latchfordi* is

TABLE II

Fauna of the sand habitat

	2*	5*	6	7*	9	10*	12*	22*	29
E. complanatus	x								x
Pisidium sp.	x						x		
L. s lillianae	x	x	x	x	x	x	x	x	x
P. p. latchfordi	x	x	x	x	x	x	x	x	
H. a. latchfordi	x	x		x		x	x	x	
A. l. porata							x		
C. ?decisum	x						x		
Musculium sp.							x		
H. c. wisconsinense	x	x	x	x	x	x			

	33*	34	36	38	41	43	44*	45	52
E. complanatus									
Pisidium sp.									
L. s. lillianae	x	x	x	x	x	x	x	x	x
P. p. latchfordi	x	x	x	x	x	x	x	x	
H. a. latchfordi	x						x		
A. l. porata									
C. ?decisum	x	x							
Musculium sp.									
H. c. wisconsinense									

**Water shallow (0–3 ft.); others, water deep (3–10 ft.).*

found abundantly. *Campeloma* cf *decisum* is probably more abundant than the table indicates, for owing to its burrowing habits it is harder to find. The other species are represented only by occasional specimens.

Fauna of the Mud Habitat

Nine stations of this type were examined. These may be divided into two groups:

A: Occasional small areas of mud, without plentiful vegetation (Stations 3, 14, 25 and 28).

B: Large areas, with plentiful vegetation (Stations 11, 11a, 16, 21 and 26).

The fauna of group A is very much like that of the rock bottom, the same two characteristic species being present. That of group B is entirely different. In the shallow bays of the lake, the bottom is covered with a dark brown, sticky, soft mud which supports a large number of water plants: white water lily *Nymphaea odorata* Ait., yellow water lily *Nymphozanthus advena* (Ait.) Fern., water milfoil *Myriophyllum alterniflorum* D.C., bulrushes *Typha latifolia* L. and perhaps also *T. angustifolia* L., pickerel-weed *Pontederia cordata* L., water-weed *Elodea canadensis* Michx.

On these weeds, as well as on the mud bottom, a large number of species were found. Their distribution is shown by stations in Table III.

TABLE III

FAUNA OF THE MUD HABITAT

	3	11	11a	14	16	21	25	26	28
Anodonta marginata		x(1)	x		x	x		x	
Gyraulus parvus			x		x	x		x	
A. ?limosa porata			x		x	x		x	
Ferrissia parallela			x		x	x		x	
H. c. wisconsinense			x		x	x		x	
H. t. pilsbryi			x		x	x		x	
Ph. gyrina			x		x	x		x	
P. columella			x		x	x		x	
L. s. lillianae				x	x(2)	x(2)	x		x
Ph. p. latchfordi	x			x			x		x
H. a. latchfordi	x								
Succinea retusa								x	

(1) *Other species present but only A. marginata taken.*
(2) *Approaching var. jugularis; one specimen at each locality. See note page 50.*

Lymnaea stagnalis is present but very scarce, and the only two specimens taken have an elongated spire, approaching that of variety *jugularis*, a response to a quiet water environment. *Physa gyrina* here replaces *P. parkeri latchfordi*. In the mud itself *Anodonta marginata* is common but it was on the underside of water lily leaves that the greatest variety of species was taken. On these the following were found: *Gyraulus parvus* (Say), *Amnicola ?limosa porata* (Say), *Ferrissia parallela* (Say), *Helisoma campanulatum wisconsinense* (Winslow), *Helisoma trivolvis pilsbryi* (F.C.B.), *Physa gyrina* Say and *Pseudosuccinea columella* (Say). The number of individuals on a leaf varied, however. Specimens were more abundant on the large, rounded leaves of the white water lily than on the narrower, pointed ones of the yellow water lily. Leaves in an advanced state of decay were devoid of specimens, as were also the healthy, green leaves. The preferred habitat seemed to be those leaves which were just turning yellow.

Bulimnaea megasoma (Say) should live on the mud, in shallow water. The conditions in some places, especially at Stations 11, 11a and 21 are exactly similar to those in Taylor Lake where the species is abundant, yet no specimens were found on the mud anywhere in Meach Lake.

Fauna of the Inlets and Outlet

For purposes of comparison the following three faunules are included here. The fauna of McGoey Lake is essentially the same as that of Meach and so is not considered further here. The fauna of the inlet, of the small creeks flowing into the lake, and of the outlet, show the effect of a different environment on the same species.

Dwarf fauna: At the outlet of McGoey Lake, Station 61, the water rushes over an exposure of granite on which some very peculiar specimens were collected. They belong to two species only, *Lymnaea stagnalis* and *Physa*

?parkeri latchfordi. It seems that this particular combination of fast running water and rock bottom is unfavorable to molluscan life and that those species which are able to live there at all can only grow a shell smaller in all dimensions than those of the lake proper. Table IV shows a comparison of specimens of the dwarf fauna with lake specimens having the same number of whorls.

TABLE IV

COMPARISON OF MEASUREMENTS, DWARF FAUNA AND LAKE FAUNA

Station	No. of whorls	Length, mm.	Width, mm.	Ap. length, mm.
Lymnaea stagnalis lillianae				
61	4½	23	13.5	13.5
23	4½	34	18	19
61	5	20.5	10.5	12
61	5	21	11	12
61	5	24	14	14.5
61	5	24	14.5	13.5
61	5	25.5	14	15
61	5	25.5	14.5	15
61	5	26	15	14.5
61	5	28.5	16	16.5
61	5	30	17	17
16	5	33	19	22
23	5	39.5	21	23
23	5	39.5	23	23
16	5	40	20	24
61	5½	27	15	16
16	5½	45.5	24	25.5
Physa parkeri latchfordi				
61	4½	15	9.5	
8	4½	19.5	12	
61	5	15.5	11	
61	5	15.5	10	
23	5	20	14.5	
10	5	20	15	
8	5	20.5	14	
8	5	21	14	
61	5½	14	10.5	
10	5½	25	18	

From the table it will be seen that the specimens of *Lymnaea stagnalis* must be put under the variety *lillianae* since in every case the aperture is longer than the spire.

The *Physa* is doubtfully referred to *latchfordi* rather than *gyrina* since some of the specimens have a slight shoulder.

Fauna of the Inlet

In the inlet the following species were collected: *Physa parkeri latchfordi* F.C.B., *Helisoma anceps ?latchfordi* (Pils.), *H. campanulatum wisconsinense* (Winslow), *Amnicola* sp., *Elliptio complanatus* (Dillw.). The *Helisoma anceps* are uniformly smaller than those from the lake but otherwise similar. The same may be said of the *Physa* and *Helisoma campanulatum wisconsinense*.

Fauna of Tilley Creek

Tilley Creek, emptying into Tilley Bay (Station 2) furnished a few specimens of an unidentified *Pisidium*. The water of the creek is much colder than that of the lake and remains so for about 20 ft. after it joins the lake. In this narrow band of cold water *Pisidia* were found sparingly but in the warmer water on each side only dead specimens could be found.

TABLE V

Occurrence of the species by habitats

	Rock	Sand	Mud	Dwarf fauna	Inlet	Tilley Creek
Musculium rosaceum		dr				
Pisidium sp.		dr				c
Anodonta marginata			c			
E. complanatus	r	r			r	
L. s. lillianae	c	c	r	c		
B. megasoma	r					
P. columella			c			
G. parvus			c			
H. t. pilsbryi	r		c			
H. c. wisconsinense		c	c		r	
H. a. latchfordi	r	c	r		r	
P. p. latchfordi	c	c	r	c	r	
P. gyrina			c			
F. parallela			c			
A. ?limosa porata		r	c		r	
Campeloma cf. *decisum*		c				
Succinea retusa			r			

r: *rare;* dr: *rare, dead specimens only;* c: *common.*

Problems Suggested

1. *Bulimnaea megasoma:* Since it is known that this species is present upstream from Meach, the lake should be carefully watched in 1935 and subsequent summers to see if it will reappear there. If so, its disappearance in 1934 can be interpreted only as a minor fluctuation; if not, then it can be concluded that conditions have become permanently unfavorable for that species.

2. *Dwarf fauna:* It would be interesting also to see whether the dwarf fauna will reappear in the summer of 1935. Dwarf specimens have been collected only once and the conditions which produced the dwarfing may have been temporary. Further collecting at Station 61 would settle this point.

3. *The Outlet:* Meach Creek, the outlet of Meach Lake, should be investigated thoroughly over the whole of its course to the Gatineau River. It is more than probable that the peculiar varieties present in the lake are carried into the creek and perhaps even into the Gatineau River; yet they have never been found in the river. An investigation of Meach Creek may throw some light on the disappearance of these varieties, or their modification into river forms.

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STUDIES OF TOTAL ERYTHROCYTE AND LEUCOCYTE COUNTS OF FOWLS

III. VARIATION IN NUMBER OF BLOOD CELLS OF NORMAL FOWL[1]

BY JACOB BIELY[2] AND E. I. PALMER[3]

Abstract

Following the technique recommended by Shaw in which the erythrocytes and leucocytes are counted in the same chamber, the authors made total erythrocyte and leucocyte counts of 100 apparently healthy and vigorous single-comb White Leghorn hens. Following the counts, post-mortem examinations did not reveal any striking abnormalities. With few exceptions the birds were singularly free from intestinal parasites.

The range of the erythrocyte counts was found to be 1,805,000 to 3,845,000, and of leucocyte counts 18,330 to 49,000. The data are analyzed statistically.

In respect to the erythrocyte counts, the authors' data are in complete agreement with the recent studies by Cook and Dearstyne of 75 female birds. There is, however, a marked difference in the range (3,000-47,000) and the mean of the leucocyte counts, which may be due to the different technique employed by Cooke and Dearstyne.

Total erythrocyte and leucocyte counts were also made of 47 one- to two-day-old chicks. The range of the erythrocyte counts was found to be 1,800,000 to 3,180,000 and of the leucocyte counts 15,000 to 28,330. A statistical analysis of the data is presented. In the case of the *baby chicks*, since the environmental conditions are relatively uniform, it would appear that factors other than environment affect the total erythrocyte and leucocyte counts of individual chicks.

Considerable variation has been noted in the total count of red and white cells of the blood of normal fowls by different investigators. Comparatively little consideration, however, seems to have been given to the influence of such factors as breed, sex, age, diet, climate and geographical habitat, on the numerical constituents of the blood. Moreover, a systematic study of the extent and degree of the variation, as in the case of other domestic or laboratory animals, has not been reported.

Table I shows a comparison of erythrocyte and leucocyte counts reported in the literature. A part of the data was taken from the excellent review by Scarborough (21) of all available recorded findings up to 1931 on the haematology of the fowl.

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[1] *Manuscript received July 13, 1935.*

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Scarborough's data, as well as additional data reviewed by the authors, are listed in Table I. Scarborough states that the average number of erythrocytes found in 246 counts by 20 investigators is 3,440,000 per cu. mm., with a normal range of from about 2,800,000 to 4,500,000. The average

TABLE I

TOTAL ERYTHROCYTE AND LEUCOCYTE COUNTS OF NORMAL FOWLS AS DETERMINED BY DIFFERENT INVESTIGATORS

Author	Year	Erythrocytes			Leucocytes		
		Ave.	Max.	Min.	Ave.	Max.	Min.
Stoltzing (21)	1856	3.9					
Malassez (21)	1872	3.1					
		2.7					
Hayem (21)	1889	2.4			26.3		
Albertoni and Mazzoni (21)	1891	2.5			32.3		
Moore (21)	1895–6	3.6	3.7	3.5	20.0	21.2	18.9
Heinz (21)	1901	4.0	4.1	3.9			
Ward (21)	1904	3.3	4.0	2.4	36.2	24.0	61.0
Warthin (21)	1907	2.5	3.0	2.0	21.0	29.0	12.0
Ellerman and Bang (21)	1908	3.0			30.0		
Hirschfeld (21)	1909	3.0			30.0		
Goodall (21)	1910	3.2			19.0		
Hedfeld (21)	1911	4.2	5.1	3.6	24.0		
Klieneberger and Carl (21)	1912	3.1				60.0	35.0
Schmeisser (21)	1915	3.5	4.0	3.0		80.0	20.0
Pickens (20)	1915–16	3.5	4.0	3.0	35.0		
Taylor (21)	1916				23.7	30.0	17.0
Fatham (21)		4.7	5.8	3.6			
Launoy and Levy-Bruhl (21)		2.98			28.0	35.0	20.0
Mack (21)		3.0			33.8		
					55.2		
Burnett (21)	1917	3.0	4.0	2.0	25.0	36.0	18.0
					17.9		
Kaupp (12)	1918	3.–4.0			28.–35.0		
Salomon (21)	1919	3.2	3.4	3.1	38.5	49.0	28.0
					49.0	57.0	41.0
Fritsch (21)	1920	3.0					
Klieneberger (14)		M 3.4–3.9			23–32.0		
		F 2.9–3.9			23–36.5		
Kennett (13)	1926	2.9			33.0		
Chaudhuri (6)	1926	M 4.6	5.2	3.9			
		F 3.1	3.8	2.0			
Blacher (1)	1926	M 3.8	4.4	3.0			
		F 2.9	3.7	2.1			
Hayden (9)	1927–8	2.8	3.7	2.3	38.9	48.0	22.0
Breusch (4)	1928	3.5			33.3		
Blain (3)	1928				18.6	29.5	10.1
						85.0	
Kyes (15)	1929					13.0	8.0
Forkner (11)	1929	3.3	4.6	2.3	24.6	74.0	6.8
Thomsen and Engelbreth Holm (24)	1931	3.0			30.000		
Fenstermacher (10)	1932	2.6	2.8	2.2	2.4	29.3	19.8
Seagar (22)	1933	2.9			27.0		
Cook and Dearstyne (7)	1934	M 3.9	4.2	3.7	14.7	19.1	9.3
		F 2.8	3.5	1.8	16.8	47.0	3.0
Landauer and David (16)	1933	M 4.3	4.6	4.0			
		F 3.4	3.7	3.2			
Gibbs (8)	1934	3.2			24.0		
Blakemore (2)	1934	2796			25.1	36.0	13.3

number of leucocytes found in 126 counts by 13 investigators is 25,900 per cu. mm. with a normal range of from about 20,000 to 40,000. The averages as given by Scarborough do not take into account the sex, age, weight, environmental conditions or geographic locations of the fowl.

It will be seen from the data presented by Scarborough that the observations of the individual authors were based on a comparatively small number of birds. A more extensive study of the haematology of the normal fowl, based on 80 normal individuals, was reported recently by Cook and Dearstyne (7).

Forkner (11) states that "an analysis of the literature reveals that most of the discrepancies in the blood counts of normal birds as given by various investigators are due to four causes. These are: study of insufficient numbers of animals; failure to make a sufficient number of observations on single animals; lack of an adequate method of counting blood cells; and confusion of thrombocytes with other elements of the blood. It must also be borne in mind that there are large individual fluctuations of the leucocyte counts of fowls which are considerably greater than those encountered in mammals."

In passing, it may be noted that up to 1928 the estimation of the number of leucocytes in the blood of chickens was based on an indirect count in which the ratio of white cells to red cells was calculated from fixed smears. Investigations conducted since 1928 for the most part are based on a direct method of counting red and white cells as well as thrombocytes.

The purpose of the present paper is to report data on the total erythrocyte and leucocyte counts of a uniform group of normal birds.

Material and Methods

The birds used in this investigation were 100 S.C. White Leghorn females. With the exception of a few individuals they were not in laying condition, since they had just completed their first egg laying year and were approximately 20 months of age. The 100 females were secured from different farms and kept together for about one month in one laying house before the blood counts were begun. Mash, grain and water were before the birds at all times.

Owing to the season of the year (October-November) the birds were moulting, some being in an early, while others were in an advanced stage of the moult. Throughout the period of observation the birds appeared to be healthy and vigorous.

The method and technique adopted in making the total erythrocyte and leucocyte counts were those described previously (17, 18). The method was that recommended by Shaw (23). An attempt was made to maintain conditions that were as nearly uniform as possible. Blood dilutions of the first 40 birds were made with one pipette, while the blood of the remaining 60 birds was diluted with another pipette. The same counting chamber was used for all the counts.

A record was made of the room temperature, the time of day the count was made, the weight of the bird, and the number of red and white blood cells counted on each side of the counting chamber. The counts of the 100 birds were completed within about three weeks. Total erythrocyte and leucocyte counts were made also of 47 one- to two-day-old S.C. White Leghorn chicks later in the season.

TABLE II

ERYTHROCYTE AND LEUCOCYTE COUNTS OF 100 S.C. WHITE LEGHORN HENS

No. of Bird	Erythrocytes (000)	Leucocytes	No. of Bird	Erythrocytes (000)	Leucocytes
1	3,175	18,880	51	2,425	38,440
2	2,145	24,550	52	2,490	44,770
3	2,925	29,770	53	2,630	34,880
4	2,985	29,770	54	2,600	34,220
5	3,260	34,880	55	2,730	36,330
6	3,310	34,330	56	2,470	26,440
7	2,615	18,330	57	2,705	35,550
8	3,235	34,000	58	2,750	32,220
9	2,850	35,880	59	2,710	36,000
10	2,800	36,000	60	2,725	32,110
11	2,585	40,660	61	2,945	37,550
12	2,560	30,660	62	2,615	30,220
13	2,550	19,000	63	2,475	22,550
14	2,725	46,220	64	2,505	24,440
15	3,155	32,330	65	2,980	30,220
16	2,955	43,000	66	2,405	28,000
17	2,765	30,000	67	2,530	25,880
18	2,775	38,440	68	2,315	30,880
19	2,685	34,000	69	3,415	38,660
20	3,845	35,880	70	2,590	23,000
21	3,205	25,330	71	2,315	30,110
22	2,305	39,880	72	3,210	49,000
23	3,110	37,880	73	2,760	29,330
24	2,880	31,880	74	2,570	40,110
25	2,770	23,440	75	3,025	33,000
26	2,780	32,770	76	2,390	34,660
27	3,160	32,330	77	2,600	30,330
28	2,920	22,880	78	3,340	20,330
29	2,715	22,550	79	2,575	33,220
30	3,490	30,770	80	2,800	39,660
31	3,570	35,000	81	2,990	29,000
32	2,375	20,000	82	2,930	35,770
33	2,885	34,000	83	2,450	23,880
34	3,020	37,220	84	2,140	40,440
35	2,905	36,660	85	2,975	30,660
36	2,730	29,220	86	3,265	47,000
37	2,675	34,440	87	2,800	33,880
38	2,435	34,330	88	2,645	23,000
39	2,505	41,000	89	2,925	31,440
40	2,690	33,110	90	2,810	33,880
41	2,395	31,550	91	3,050	21,550
42	2,820	38,000	92	2,880	29,440
43	3,055	29,660	93	2,545	34,110
44	2,685	28,550	94	3,010	33,000
45	2,760	32,550	95	3,070	23,660
46	3,130	25,660	96	2,575	25,110
47	2,485	26,220	97	2,775	41,880
48	2,425	38,880	98	3,060	32,330
49	1,805	25,000	99	3,365	45,770
50	2,475	32,000	100	3,250	26,440

Data

Detailed data pertaining to the erythrocyte and leucocyte counts of 100 single-comb White Leghorn females are shown in Table II. It will be seen that the mean erythrocyte count was 2,782,000 per cu. mm. and the mean leucocyte count was 32,150 per cu. mm. The range in the erythrocyte counts was 1,805,000 to 3,845,000 and that of the leucocytes 18,330 to 49,000.

Tables III and IV show the frequency distribution of the erythrocyte and leucocyte counts respectively. This is strikingly illustrated in Figs. 1 and 2.

TABLE III

FREQUENCY DISTRIBUTION OF THE ERYTHROCYTE COUNTS OF 100 S.C. WHITE LEGHORN HENS

No. of erythrocytes (000)	Under 1999	2000–2249	2250–2499	2500–2749	2750–2999	3000–3249	3250–3499	3500–3749	3750–3999
Percent. of total birds	1	2		28	28	15	8	1	1

TABLE IV

FREQUENCY DISTRIBUTION OF THE LEUCOCYTE COUNTS OF 100 S.C. WHITE LEGHORN HENS

No. of leucocytes	Under 19,999	20,000–24,999	25,000–29,999	30,000–34,999	35,000–39,999	40,000–44,999	45,000–49,999
Percent. of total birds	3	13	17	37	19	7	4

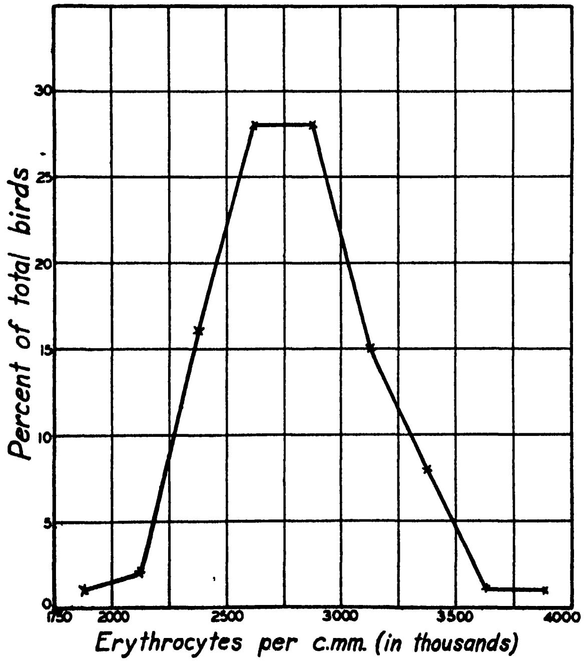

FIG. 1

TABLE V

Statistical analysis of erythrocyte and leucocyte counts

	Authors' data on 100 ♀ birds		Cook and Dearstyne data on 75 ♀ birds		Authors' data on 1- to 2-day-old chicks	
	Erythrocytes	Leucocytes	Erythrocytes	Leucocytes	Erythrocytes	Leucocytes
Mean	2782000 ± 22580	32150 ± 450	2760000 ± 26578	16800 ± 726	2480000 ± 31576	20600 ± 286
Standard deviation	334777 ± 15969	6680 ± 318	338885 ± 18735	9265 ± 509	318338 ± 22366	2889 ± 202
Coefficient of variability	12.03 ± 0.58	20.77 ± 1.02	12.27 ± 0.67	55.09 ± 3.80	12.83 ± 0.91	14.02 ± 0.99

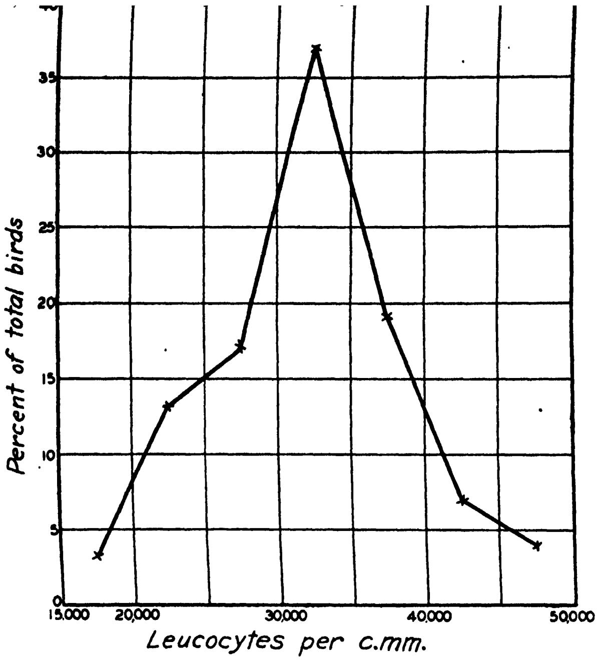

FIG. 2

The data presented in Table II were analyzed statistically by the usually accepted formulas (17). Determination of the following numerical values for the total red and white cells was made: mean counts, the probable error of the mean, standard deviation and its probable error, coefficient of variation and its probable error. (Table V).

The average number of red cells counted on each side of the chamber was 281.24 and 279.14 or a difference of 2.10 cells and of white cells 142.41 and 145.15 or a difference of 2.74 cells. The difference between the number of cells counted on both sides of the chamber, according to class ranges is shown in Table VI. In the great majority of cases there was very little difference in the number of cells counted on both sides of the chamber, indicating that the actual counting was performed with great accuracy. As a matter of fact the actual difference was negligible since in each case the average of the two counts was taken.

TABLE VI

DIFFERENCE IN THE NUMBER OF CELLS COUNTED ON BOTH SIDES OF THE CHAMBER

Range in number of cells	0–5	6–10	11–15	16–20	21–25	26–30
Erythrocytes, No. of birds	26	40	26	7	1	
Leucocytes, No. of birds	34	32	26	2	2	4

The erythrocyte and leucocyte counts of the 47 one- to two-day-old chicks are shown in detail in Table VII. The mean erythrocyte count was 2,480,000 per cu. mm. and the mean leucocyte count was 20,600 per cu. mm. The range in the erythrocyte counts was 1,800,000 to 3,180,000 per cu. mm. and in the leucocyte counts 15,000 to 28,300 per cu. mm. A statistical analysis of the data is shown in Table V.

TABLE VII

ERYTHROCYTE AND LEUCOCYTE COUNTS OF 47 ONE- TO TWO-DAY-OLD S.C. WHITE LEGHORN CHICKS

No. of bird	Erythrocyte (000)	Leucocyte	No. of bird	Erythrocyte (000)	Leucocyte
1	2885	18,770	25	2030	17,440
2	2580	21,550	26	2525	23,220
3	2745	17,440	27	2830	18,770
4	3050	20,110	28	2540	22,110
5	2625	23,000	29	2595	17,000
6	2030	15,000	30	2175	15,440
7	2480	23,440	31	2265	16,220
8	2845	23,550	32	2315	19,880
9	3180	22,000	33	2430	18,000
10	2515	25,110	34	2265	22,880
11	2635	15,220	35	2345	20,550
12	2590	22,440	36	2695	22,220
13	2390	21,660	37	2265	23,660
14	2170	19,550	38	2955	15,000
15	2900	22,660	39	2250	15,660
16	2230	23,550	40	2045	17,770
17	1800	19,550	41	2110	28,330
18	2835	20,330	42	2065	22,880
19	2710	23,110	43	2410	24,660
20	2545	16,440	44	2320	22,330
21	2010	15,000	45	1910	20,550
22	2095	20,880	46	2980	15,330
23	2610	23,110	47	2365	19,880
24	2240	21,770			

Considerable difficulty was at first experienced in obtaining accurate blood cell counts of baby chicks. Extreme care had to be exercised in securing the blood and mixing it immediately with the diluting fluids.

Discussion

The first problem that confronted the authors in this investigation was the question of the "normality" of the birds. Observations based on physical appearances indicate that so-called normal birds may show various degrees of internal pathological involvement. Suffice it to recall that pullorum disease "carriers" which appear to be normal nearly always show a diseased condition of the ovary and sometimes of the heart.

In order to gain some idea about the normality of the 100 birds used in this investigation, every bird was killed and a detailed post-mortem examination carried out. The condition of the intestine was carefully examined and a record made of any coccidia or other intestinal parasites found.

With few exceptions the internal organs appeared to be normal. Two birds were found with only one kidney each. The intestines were singularly free from parasites and no coccidia could be detected in the scrapings of the intestinal tract. The liver, spleen and kidneys of each bird were weighed and with few exceptions appeared to be normal in size, texture, and color. All other organs appeared normal. The healthy condition of the birds may be attributed to the fact that only the most vigorous birds had been retained as a result of rigorous culling of the flock during the laying year. A heavy infestation of round worms and tape worms was found in two birds. In the case of a few birds the ovary showed slight pathological involvement and *S. pullorum* was isolated. It may be noted that in these cases the erythrocyte and leucocyte counts did not deviate from the average counts of the whole group.

It may be assumed from the post-mortem examination that the birds used in this investigation were as near normal as could be expected of birds kept under ordinary commercial conditions. As far as the authors are aware, this study is the only one in which examination of the internal organs was made immediately following the taking of the blood counts.

The data presented in this investigation fall within the range of the minimum and maximum values reported by previous investigators. An examination of Figs 1 and 2 and Tables III and IV shows that the frequency distribution of the erythrocyte and leucocyte counts approached a normal curve. It is significant that the probable error of the mean, of the standard deviation and of the coefficient of variation is in each case low.

The coefficient of variability which represents an absolute measure of variability is in the case of the erythrocytes 20.77 ± 1.02 and in the leucocytes 12.03 ± 0.58. It may be noted that in a statistical study of the variation in the blood counts of 100 normal rabbits, Bushnell and Bangs (5) reported a coefficient of variation of 13.01 in the erythrocyte and 20.83 in the leucocyte. Similarly Pearce and Casey (19) showed that the coefficients of variation of erythrocytes and leucocytes, based on 1110 counts of 174 normal rabbits, were 12.09 and 20.53 respectively.

Since the studies of Cook and Dearstyne (7) are the only ones based on a large number of birds, comparable in numbers to ours, it seems worth while to compare our results with theirs. To facilitate analysis the data of both investigations are shown in Table V.

It will be seen that the mean, minimum and maximum of the erythrocyte counts reported by Cook and Dearstyne correspond closely with those reported in this investigation. The frequency distribution in the different classes is practically the same.

With regard to the total leucocyte counts there appears to be considerable disagreement between the data presented in this paper and those of Cook and Dearstyne. (Range 3000–47,000). A statistical analysis made of the data presented by Cook and Dearstyne (Table V) shows that the coefficient of variability of the erythrocytes was comparatively low while that of the leucocytes was very high.

The wide divergence found between the leucocyte counts reported by Dearstyne and by the writers may be due to differences in technique or to the physiological condition of the birds employed in the two investigations. The high coefficient of variability (55.09 ± 3.80) of the leucocyte counts of Cooke and Dearstyne's birds suggests that their group of birds may not have been as uniform physiologically as the present series (coefficient of variability 20.77 ± 1.02). As regards technique, that used by the authors was Shaw's (23) while that used by Cook and Dearstyne was Forkner's (11). In the latter technique, both the direct and indirect method are involved, while in the former, the direct method only is used.

It would seem from a consideration of the data presented in this investigation that no true comparison can be made between the results of one investigation and those of another, until more uniform methods of making total leucocyte counts are adopted.

The data show that considerable variation has been found in the erythrocyte and leucocyte counts of a fairly uniform group of birds such as recorded in this paper. The variation in the leucocyte counts was greater than that of the erythrocyte counts. It is of interest to note that the mean erythrocyte and leucocyte count of the one- to two-day-old chicks was lower than that of the adult birds and that the difference was statistically significant. The standard deviation and coefficient of variability were much lower in the one- to two-day-old chicks than in the adult birds. In the case of the baby chicks, since the environmental conditions were relatively uniform it would appear that factors other than environment may affect the total erythrocyte and leucocyte counts of individual chicks.

Aside from the question of standardized technique and uniformity of the subjects under investigation, there may be genetic factors that influence the total red and white count of various families or strains of birds kept under the same conditions. A study of the genetic aspects of the question might, in part, account for some of the variations noted in this and other investigations.

Acknowledgment

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THE WHITE APPLE LEAFHOPPER (*TYPHLOCYBA POMARIA* McA.)[1]

By Thomas Armstrong[2]

Abstract

Typhlocyba pomaria McA., the white apple leafhopper, a pest of apple in Canada and the United States, has two generations a year in the Vineland district of Ontario. The insect spends the winter in the egg stage, the newly hatched nymphs make their appearance in May, and adults are present on the trees in June and July. Summer eggs are deposited chiefly in the midribs of the foliage, and take from 20 to 64 days to hatch. The second generation of adults matures in August and September. Detailed information on all phases of the life history is presented, including the type of feeding and nature of the injury produced on apple foliage. The associated species of leafhoppers which cause injury to apple are listed.

Introduction

The species *Typhlocyba pomaria* McA., until 1926, was confused with other species of leafhoppers attacking apple. In the main the insect has been taken for *Typhlocyba rosae* (L.) in American and Canadian literature, and it is altogether likely that publications dealing with the rose leafhopper as an apple pest, before 1927, may apply, in part at least, to *T. pomaria.* McAtee (12) first differentiated the two species in 1926, and gave a description of *T. pomaria.* Ackerman (1), in 1931, proposed the common name "white apple leafhopper" for this apple pest, and this name was approved by the American Association of Economic Entomologists.

In 1931, DeLong (6) wrote the following with regard to the confused status of the leafhoppers attacking apple. "*T. pomaria* is the most common and wide-spread species of this genus occurring on apple. It is apparent that previous records of *rosae* in abundance are in error, and observations in areas where *rosae* was previously reported have shown either *pomaria* or *xanthippe* to be the important species. The species reported by Childs (5) as *rosae* is apparently *pomaria* since that is the important species of *Typhlocyba* occurring in that area (Oregon) according to recent observations."

T. pomaria has been present in Ontario apple orchards for a considerable number of years. In 1919 it was reported (14) that myriads of leafhoppers were present in many apple orchards in the Niagara district, the species at that time being called *Empoa rosae.* Mr. W. A. Ross* is of the opinion that this species was really *pomaria.* It would appear that there was a bad infestation in Ontario during the seasons of 1919 and 1920, followed in 1921 by a considerable reduction in leafhopper population brought about by natural agencies. In 1927, the white apple leafhopper was very abundant, especially in the counties of Prince Edward, Northumberland, Durham, Peel, Went-

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worth, Lincoln and Norfolk. It was sufficiently abundant in 1931, in the Niagara district and western Ontario, to cause conspicuous mottling of the foliage late in the season, and in 1932 some orchards were very heavily infested. Mr. Ross reports the species as occurring in British Columbia, and Gilliatt (14) records it as present in Nova Scotia apple orchards, and being particularly numerous in 1930 in the Berwick district. It was prevalent and widespread in Annapolis Valley orchards during 1931.

In the United States the species is widely distributed, being known to occur in the states of New York, Connecticut, Pennsylvania, Virginia, Arkansas, Oregon and Idaho. In all probability *T. pomaria* will be found in most of the apple growing districts.

The information contained in this paper was compiled from original notes taken during the years 1933 and 1934. The work was carried out at Vineland Station, Ontario.

The First Generation

OVERWINTERING EGG

Description

The egg is white, elongate, cylindrical and slightly tapering at the ends, which are quite blunt. The anterior end, that portion deposited last, has a noticeable curve upwards to the surface, tending to form a channel. The average length of the egg is .80 mm., and the width, .26 mm.

Location

The overwintering eggs are laid singly beneath the bark in the collenchyma tissue of the cortex. In most cases they are situated crosswise to the axis of the stem, and appear to the eye as raised areas, or small blisters, quite regular in outline. The egg itself lies practically horizontal, with possibly a slight slope down towards the posterior end.

Distribution on the Apple Limb

An apple limb was examined on April 19, 1933, to see where the leafhopper eggs were laid. Table I gives the results.

TABLE I

Year wood formed	Length of growth, in.	Number of fresh eggs	Remarks
1932	9	0	—
1931	8	5	—
1930	13	45	—
1929	5	53	Numerous old egg blisters
1928	4	52	Numerous old egg blisters
1927	5	49	Numerous old egg blisters
1926	4	23	Numerous old egg blisters
1925		Fresh eggs present	
1924	—		Numerous old egg blisters

Process of Hatching

When hatching takes place the "head" end of the slightly concave side of the blister shows a break in the bark tissue, from which the embryo protrudes. The nymph, as it slowly appears, breaks its protecting sheath and slides forth, leaving the egg shell thrust well out of the old egg cavity.

TABLE II

DURATION OF HATCHING OF FIRST GENERATION EGGS
(Cages in McKenzie's Orchard)

Date	Position of cage on tree				Total
	North	South	East	West	
May 9	9	5	1	3	18
10	7	14	9	17	47
11	51	42	11	21	125
12	8	14	15	7	44
13	28	21	30	24	103
14	27	22	30	25	104
15	23	41	33	31	128
16	16	17	30	19	82
17	24	22	33	18	97
18	34	18	55	29	136
19	29	14	35	11	89
20	12	5	11	4	32
21	12	4	12	4	32
22	9	7	8	5	29
23	4	4	7	3	18
24	2	2	1	2	7
25	2	2	1	2	7*
26	4	3	3	1	11*
27	3	3	4	0	10*
28	2	3	3	1	9*
29	4	4	16	2	26*
30	7	2	4	4	17*
31	4	2	2	9	17*
June 1	3	2	3	1	9*
2	0	0	2	4	6*
3	0	0	0	1	1*
4	0	0	0	0	0*
5	0	1	0	0	1*
6	0	0	0	0	0*
Totals	324	274	359	248	1205

* *E. maligna nymphs in part.*

Duration of Hatching Period

In 1933, overwintering eggs commenced hatching on May 13, at which time the apple cluster buds were separating. Newly hatched nymphs could be found in the orchards until the end of May. The following year, 1934, eggs were hatching from May 9 to June 1. Table II shows the duration of the hatching period, as well as the time when the largest number of nymphs appeared. Insectary rearing records showed that the last eggs hatched on June 1, the date on which the calyx spray was applied in the apple orchard. The percentages of the total nymphs to appear at various times during the hatching period were as follows:

May	9	First nymph
	15	46.4% of eggs hatched
	22	88.5% of eggs hatched
June	1	100.0% of eggs hatched

Thus two weeks after the first egg hatched practically 90 per cent of all the eggs had produced their nymphs.

THE NYMPH

Description

This insect passes through five nymphal stages, moulting five times. As moult follows moult the leafhopper gradually increases in size and the wings develop. A short description of each instar is given herewith.

First instar. Color pale white to light yellow, with eyes a dull red. The first two segments of the antennae are pale, the remainder dusky. Size, .890 mm. long, and .217 mm. wide.

Second instar. Color varies from creamy white to light yellow. Wing pads present. The posterior margin of the metathorax is definite in outline, and is the main character which separates this instar from the first and third. Eyes not as red as in first instar. Size, 1.156 mm. long, and .320 mm. wide.

Third instar. Color as in second instar. Body is stouter, with the wing pads extending to hind margin of first abdominal segment. Eyes a dull white in color. Size, 1.650 mm. long, and .400 mm. wide.

Fourth instar. Color dull white to a distinct yellowish. Eyes whitish in color. Wing pads extend to hind margin of second abdominal segment. Size, 1.970 mm. long, and .490 mm. wide.

Fifth instar. Color light yellow to a more distinct yellow. Eyes distinctly pearl white. Wing pads extend to the fourth abdominal segment. Size, 2.610 mm. long, and .686 mm. wide.

Habits

The first generation nymphs, hatching on the young wood as they do, have to wander off in search of foliage for feeding purposes. Very shortly after emergence the small ungainly creatures become very active, especially if the day is warm, and migrate rapidly along the limbs, spurs and petioles until the underside of the leaf is reached. Here, feeding is carried on. The nymphs confine their attack mainly to the lower parts of the trees, and they are never to be found on the terminal *growing* shoots. Very rarely does one find nymphs migrating, and it is the opinion of the writer that they remain on a satisfactory leaf until maturity. Only the fifth instar nymphs are capable of hopping and this they do but rarely. Even if disturbed persistently they will not always hop away, but will use their legs in the normal manner.

Duration of Nymphal Life.

Using potted apple seedlings under lantern globes in the open insectary, the length of the nymphal life, as well as the duration of each instar, was determined from 66 individuals in 1933, and 121 in 1934, as is shown in Table III.

TABLE III

Period	Maximum length in days		Minimum length in days		Average length in days	
	1933	1934	1933	1934	1933	1934
First instar	7	12	3	3	4.65	6.66
Second instar	6	9	3	3	4.10	6.16
Third instar	5	6	2	2	3.94	4.03
Fourth instar	6	7	2	2	4.40	3.95
Fifth instar	10	8	3	5	5.83	6.64
Total life	27	37	19	19	23.0	27.45

Casting of Nymphal Skin

Moulting always takes place on the underside of the leaves, and requires a period of from 15 to 30 minutes. When the time arrives, the insect sets its claws in the leaf tissue and also inserts the mouth parts. It remains quiet for a short time, and then the skin splits along the mid-dorsal line from the head to the prothorax. The nymph pushes itself forward gradually, and works its way out, leaving the old skin attached to the leaf. The first four nymphal cast skins are not fastened to the leaf very securely for they blow away readily with the lightest breeze, but that of the last moult very often remains on the foliage all summer.

When the adult leafhopper emerges from the last nymphal moult the wings rapidly expand to their full size, the outer wings developing a little faster than the under wings. The winged insect does not fly away immediately on reaching maturity but rests very quietly for several hours close to where the moulting occurred.

Time of Occurrence of First Brood Nymphs

Nymphs (Plate I Fig. 1) were present in the orchards from May 13 to June 22 (from the time the cluster buds were separating to the time when the fruits of McIntosh and Greening were slightly more than one inch in diameter), though by June 15 very few nymphs could be found, the greater proportion having attained maturity. In 1934, the nymphal population was at its peak during the first week of June.

In order to determine the nymphal population in the orchard in the spring of 1933, leaf clusters or single leaves were picked at random in equal numbers from the north, south, east and west sides of the trees, and the number of insects on this foliage was recorded. Counts were made daily from May 14 to May 20 inclusive, that is from the time of first appearance until 85% of the nymphs had hatched. The clusters chosen were from egg-bearing wood, and were thus most likely to possess nymphs. By May 19 the clusters were getting too large to examine thoroughly, so a single leaf was used. There

TABLE IV

POPULATION STUDIES OF LEAFHOPPERS, SPRING, 1933

Date	Number of clusters or leaves examined	Number of nymphs taken on the				Total nymphs	Average per cluster
		North	South	East	West		
May 14	200 clusters	97	21	78	89	285	1.43
May 15	200 clusters	209	78	75	102	464	2.32
May 16	200 clusters	42	37	28	107	214	1.07
May 17	180 clusters	143	203	240	129	715	3.97
May 18	100 clusters	202	495	110	132	939	9.39
May 19	120 leaves*	69	100	66	63	298	2.48*
May 20	100 leaves*	43	86	44	55	228	2.28*
		805	1020	641	677	3143	

* *Average per leaf. Equal to about 12 nymphs per cluster.*

would be, on the average, approximately five such leaves per cluster, so that the figures given for the counts made on May 19 and 20 should be multiplied by 5 to make them comparable with the earlier records (Table IV).

The Adult

Description

McAtee's (12) description is as follows:

"*Male.* Head and thorax pale yellow, more or less tinged with orange to reddish on front of vertex; tegmina hyaline, the costa, commissure and sectors yellow, apical cells faintly dusky; underparts pale yellowish, the face more or less tinged with orange to reddish, touches of this higher coloration elsewhere also on some specimens.

Hypopygium: Strongly distinguished by the falcate process forming the posterior border of the side of the 9th segment, which is heavily chitinized and black in colour; as the correlation of this falcate process with the type of aedeagus here illustrated for this species has thus far been constant, it results that the species can be recognized from the superficial aspect of the hypopygium, the black tips of the falces being easily seen. Near the upper posterior border of the falx are several backwardly directed bristly hairs; similar but shorter hairs form a row on the outer clasper, and longer softer hairs spring from the under side of this clasper, from the lower border of the 9th segment near articulation of outer clasper, and from the end of the anal tube. Outer claspers as seen from below depressed interiorly so that together they enclose a long narrow sunken area, clasper as a whole narrowed on posterior third, the apices somewhat spatulate and divergent; seen from side the outer clasper is curved upward at the beginning of its posterior third, in almost a right angle, apex somewhat spatulate and recurved; inner clasper slender, outcurved apically; the aedeagus as seen from above with a median and two lateral branches, the former considerably shorter than the latter, furcate at the tip, and reinforced along the lower surface by two longitudinal stripes extending nearly to the apex appearing as ribs on the back of aedeagus as seen from above; lateral processes becoming very slender apically the terminal portion curved outward and then forward. Aedeagus measured along curve, fully twice as long as connective.

Female. Paler than male, general coloration stramineous, with little or no yellow, orange, or reddish washing; tip of ovipositor sheath black. Eighth sternite moderately pointed medially and emarginate laterally.

Length, 3–3.5 mm."

Pre-oviposition Period.

The pre-oviposition period of 12 females of the first generation confined in lantern globe cages in the insectary in 1933, varied from 10 to 19 days, the average being 13.4 days. The following year, for seven females, the period averaged 9 days. The much shorter period in 1934 was due, no doubt, to the more sunny surroundings of the open insectary where the cages rested, compared to the very sheltered conditions of the old insectary used in the 1933 work. (Table V).

TABLE V

1933			1934		
Date female matured	Date first egg laid	Pre-oviposition period, days	Date female matured	Date first egg laid	Pre-oviposition period, days
June 8	June 20	12			
9	22	13			
9	22	13			
9	25	16			
9	20	11			
9	28	19	June 9	June 18	9
9	27	18	12	21	9
10	21	11	13	21	8
10	21	11	15	25	10
12	22	10	19	29	10
12	25	13	20	28	8
12	26	14	23	July 2	9
		13.4			9

Reproductive Capacity

Records were kept of the number of viable eggs deposited by 22 females under insectary conditions. These individuals were enclosed in lantern globe cages or in small celluloid cages with a vigorous plant. One or two females were used per cage. The 22 individuals laid a total of 1174 eggs or an average of 53.4 eggs per female. Fifteen of these leafhoppers, left undisturbed throughout their oviposition period, laid an average of 62 eggs each, with a maximum of 104. In 1933, two females caged with two males when in copula on June 20, laid a total of 265 eggs or an average of 132 per female.

Period of Oviposition.

The period of oviposition varied from 7 to 34 days, as determined from the records of 15 individual females in confined quarters under insectary conditions. Egg laying extended over a period of 20.6 days on the average.

Post-oviposition Period

Female leafhoppers lived from 1 to 26 days after depositing their last fertile egg. The average post-oviposition period was 6.2 days.

Length of Life of Adults

Males survived in insectary cages anywhere from eight days to three months, the average life being 37.7 days in 1933; and 33.3 days in 1934. Females lived from 19 to 78 days, averaging 36.7 days in 1933, and 40 days in 1934. From observations in the orchard it would appear that the length of life of the insects approximates that secured under confined conditions. One leafhopper, a male, survived for 90 days.

Presence of Adults in the Orchard

The first mature apple leafhoppers were observed in the orchard on June 7, and two days later, approximately 12 days after the calyx spray, they were quite numerous. Males and females were noticed in copula for the first time on June 13. From this time until the end of July they were common in the trees. Some few could be found during the first days of August, but by this time the second generation adults had commenced to mature. Judging from the insectary rearings there is a little overlapping of the broods of adults. The last first-generation leafhopper under observation died on August 14, in 1933, and on September 7 in 1934.

For a period of two weeks starting from the time leafhoppers commenced to appear in the spring, daily orchard collections were made, and the individuals sexed. The data appear in Table VI.

TABLE VI

Date of collection	Number collected	Males	Females	Males, %	Females, %
June 6	14	14	0	100.0	0
7	28	28	0	100.0	0
8	90	90	0	100.0	0
9	56	53	3	94.6	5.4
11	84	66	18	78.5	21.5
12	61	43	18	70.5	29.5
13	74	31	43	41.9	58.1
14	63	28	35	44.5	55.5
15	72	18	54	25.0	75.0
16	88	27	61	30.7	69.3
18	78	26	52	33.3	66.7
19	60	11	49	18.3	81.7

It will be noted that during the first three days of adult emergence not a single female was collected in the orchard; and that females gradually came into the ascendency, becoming predominant in numbers one week later.

Habits of Leafhoppers

On reaching the adult stage leafhoppers are to be found resting on the under sides of the foliage. On warm days they fly very actively and, if at all abundant, can be very annoying when disturbed, for they swarm around in a cloud, and land in the hair, ears and eyes, causing some discomfort. On cool days they can be caught quite easily in vials by hand.

Mating couples were seen most frequently in the morning. The male rests with the tip of his abdomen joined to that of the female, with their heads in opposite directions, forming a straight line. The two leafhoppers remain in this position a considerable time, one pair under observation staying in copula for an hour and a half.

The Second Generation

THE EGG

Location

The eggs of this generation are deposited in the under surfaces of the leaves of apple, especially along the main ribs and large veins, and quite often in the petioles. They are practically impossible to locate prior to hatching, and even after the nymph has emerged, only a tiny scar indicates where the egg was placed. If the egg is parasitized the parasite emergence hole is readily seen, and in this way the locating of the place of deposition is made quite simple.

Time of Laying

Under insectary conditions the first eggs were deposited during the third week of June,—June 20 in 1933 and June 18 in 1934—12 days after the appearance of the first leafhoppers in the orchard. In 1934, egg laying continued into August, but the majority of the eggs were laid during the month of July. A total of 2266 eggs laid in the insectary cages were deposited at the following rate:

June 18	First egg laid
30	19.6% of eggs laid
July 15	56.2% of eggs laid
31	99.0% of eggs laid

Period of Incubation

The incubation periods of 2266 eggs varied from 20 to 64 days; the average being 39 days (Table VII).

TABLE VII

Length of egg period, days	No. of eggs	Length of egg period, days	No. of eggs	Length of egg period, days	No. of eggs
20	2	35	62	50	53
21	59	36	106	51	44
22	25	37	107	52	53
23	28	38	69	53	31
24	62	39	75	54	33
25	26	40	135	55	26
26	47	41	76	56	15
27	42	42	84	57	18
28	42	43	99	58	7
29	59	44	63	59	6
30	56	45	104	60	5
31	61	46	61	61	5
32	34	47	99	62	2
33	98	48	69	63	0
34	70	49	47	64	1
					2266

In studying the periods of incubation of summer eggs it was noticed that the first eggs deposited took the longest time to hatch, and that there was a gradual scaling down in the length of the incubation period the later the eggs were laid. For instance, the 107 eggs laid on June 28–29 averaged 45.8 days for incubation, while the 47 eggs laid on July 29–30–31 only took 26.8 days. Table VIII clearly illustrates this rather unusual phenomenon. The mean daily temperature is included only to illustrate that the shortening of the period does not depend on increases in temperature.

TABLE VIII

Date eggs laid	No. of eggs	Period of incubation, days			Mean daily temp.
		Maximum	Minimum	Average	
June 20–21	46	60	37	50.8	70.5
26–27	47	61	27	46.3	70.7
28–29	107	59	25	45.8	70.9
July 4– 5	29	60	29	43.2	70.1
10–11	107	57	27	41.8	70.1
12–13	83	54	28	39.4	69.8
14–16	130	51	29	37.8	69.8
17–19	143	48	26	36.2	66.0
20–22	104	53	23	34.3	68.9
23–25	208	42	21	27.8	69.2
26–28	217	47	20	31.8	67.8
29–31	47	37	21	26.8	67.8
Aug. 1– 3	10	32	24	29.1	66.2
6– 8	9	28	22	25.1	65.8

Duration of Hatching Period

In 1933, newly hatched nymphs were found for the first time on July 17, and, according to the insectary records, the last egg hatched on September 7. In the orchard, hatching of eggs was most active during the first three weeks of August.

The duration of the hatching period was more extended in 1934. Starting on July 17, newly hatched nymphs were taken from the foliage daily until September 28, but those appearing after September 7 were few in number.

THE NYMPH

Duration of Instars

By rearing individuals on potted plants in the open insectary it was possible to determine the length of the five instars. The information is presented in Table IX.

TABLE IX

Period	Maximum length in days		Minimum length in days		Average length in days	
	1933	1934	1933	1934	1933	1934
First instar	8	8	2	2	4.2	3.93
Second instar	6	9	2	2	3.5	3.60
Third instar	6	9	2	2	3.8	3.92
Fourth instar	7	25	3	3	4.2	4.95
Fifth instar	9	27	4	4	6.6	7.99
Total life	29	55	17	16	22.3	23.85

Time of Occurrence

In the orchard, nymphs first appeared on July 17 and gradually increased in numbers as the season advanced. By August 2, nymphs in all five stages could be found on the foliage in small numbers. Three weeks later, August 21, they were very abundant in the McKenzie orchard. After the first week in September the nymphs rapidly decreased in quantity until by September 29 they were extremely scarce.

THE ADULT

Time of Occurrence

In 1933, the first leafhopper matured on August 3, in the insectary, and adults were common in the orchard by August 15, appearing in increasing numbers from this time until well into October. On October 30, after severe frosts, the following note was recorded: "Adult leafhoppers are still plentiful in the McKenzie orchard although large numbers are dead. Dead and sluggish hoppers are numerous on the soil beneath the trees. Females still seem to be actively laying as plenty were seen on the limbs with extended ovipositors as if preparing (or just concluding) to lay." Very severe frosts occurring during the first two weeks of November no doubt brought about the death of the remaining leafhoppers.

Period of Egg Deposition

Allowing for a pre-oviposition period of about 14 days it would seem that the first eggs were deposited about August 20, in 1933. Examination of apple limbs in September showed that eggs were present in the bark of wood one-half inch in diameter. In 1934, the first overwintering eggs were deposited about August 15, a few days earlier than in the preceding year.

Proportion of Sexes

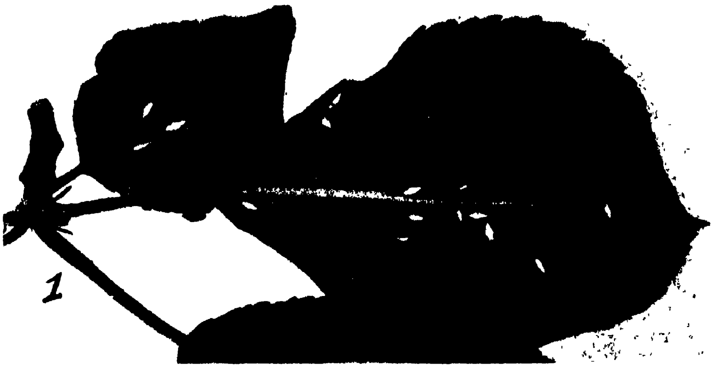
Of 157 leafhoppers reared in the insectary 57% were females. From observations in the orchard, females appeared to be more abundant than males.

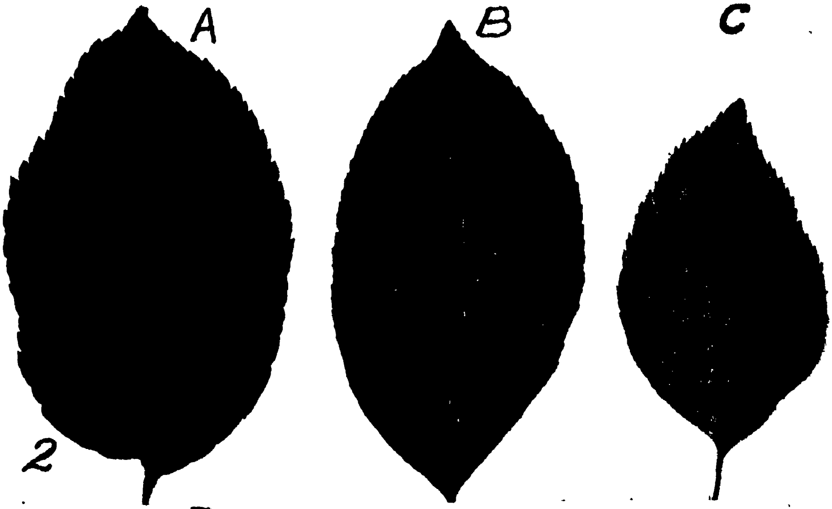
Feeding and Nature of Injury

The nymphs and adults of *T. pomaria* feed exclusively on the older leaves of apple and never attack the *growing* terminal shoots. They gather on the lower surfaces of the leaves, and, using their sucking mouth parts, extract the plant juices. The first indication of their presence is a slight mottling on the leaf's upper surface, and as the feeding is intensified the foliage loses its deep green color to take on a yellow-green appearance (Plate I, Fig. 2.). The white apple leafhopper does not feed on the fruit. To prove this, 80 adults were confined in a cage containing a single Wealthy apple. In four days every hopper had died, leaving the fruit without a blemish. In the same type of cage but with foliage substituted for the fruit, the leafhoppers lived for weeks.

The blemishing of the leaves and fruit by the excrement is very unsightly. (Plate I, Fig. 3). Rain removes a great deal of this spotting, but it may remain on the fruit long enough to hinder proper coloring.

PLATE I





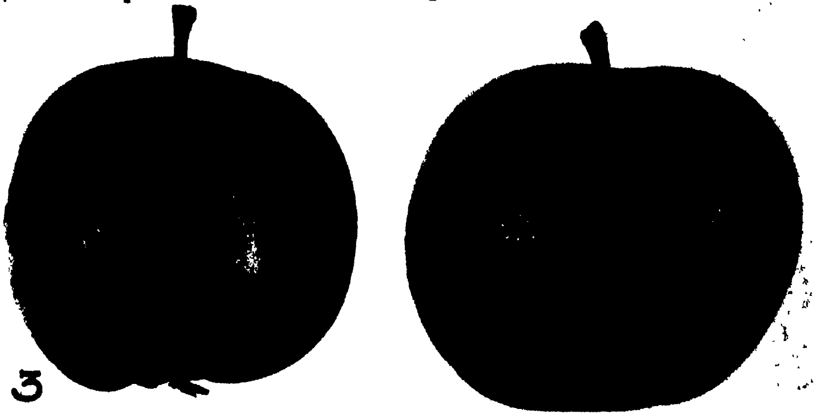
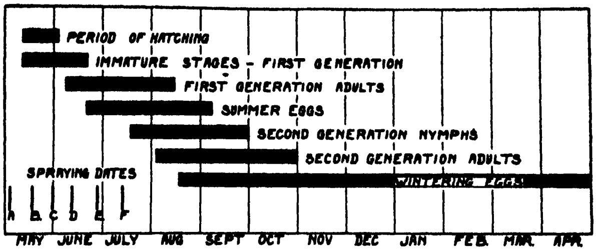


FIG. 1. *Nymphs of the white apple leafhopper feeding on apple foliage. Most of the specimens in this photograph are in the fifth instar stage.* FIG. 2. *Injury to apple foliage caused by white apple leafhoppers. A. Uninjured leaf. B. Partially injured leaf. C. Extreme injury. The small black spots in C are deposits of excrement.* FIG. 3. *Apples from a heavily infested orchard showing deposits of excrement.*

Generations

There are two generations of this insect in the year, (Text-fig 1.) with very little overlapping of any of the active stages. A few adults of the first generation are present when the early second generation leafhoppers mature. Overwintering in the egg stage, the insect first makes an appearance as a nymph in May, reaching maturity in June and July, at which time it is busy depositing eggs in the tissue of the apple leaves. The second brood of nymphs makes an appearance in July, the summer eggs taking on the average 40 days to incubate, and this in the hottest part of the season. Nymphs are numerous throughout August and most of September, and the second brood of adults can readily be found from early August to the end of October. This final brood of adults deposits eggs beneath the bark of apple limbs.



TEXT FIG. 1. *The seasonal history of the white apple leafhopper, 1933. Spray dates: A. Delayed dormant; B. Pink; C. Calyx; D. Small apple; E. First cover; F. Second cover.*

Natural Enemies

The chief predators attacking leafhoppers are spiders and birds. Adults were very commonly observed caught in the webs of spiders during the periods when flights were in progress. The predaceous Mirid, *Hyaliodes vitripennis* Say, was present in small numbers in one fairly heavily infested orchard.

Hymenopterous parasites of eggs and adults are important enemies of the white apple leafhopper. One of these, *Anagrus armatus*, var. *nigriventris* Gir., a parasite of the overwintering and summer eggs, was very common in 1933, and no doubt was instrumental in reducing the population considerably. The adult parasite, *Aphelopus* sp., nr. *microleucus* Perkins, was also abundant, more especially attacking the first brood of adults, although a small proportion of the autumn generation of leafhoppers was parasitized as well.

Associated Species of Leafhoppers

The leafhoppers which cause injury to apple in the Vineland district, in the order of their importance, are *Typhlocyba pomaria* McA., *Empoasca fabae* Harris, *Empoasca maligna* Walsh, and *Typhlocyba rosae* Linn. The grape leafhopper, *Erythroneura comes* Say, migrates to the apple in numbers in the autumn, but is not present during the greater part of the growing season. *Erythroneura lawsoniana* Baker has been taken in small numbers in a number of apple orchards throughout the peninsula. *Empoasca fabae*, the potato leafhopper, attacks the newer growth only, curling and hopper-burning the foliage, especially on nursery stock and young trees. *Empoasca maligna* was taken on apple during the spring and summer months; this insect having one generation in the year. The rose leafhopper was present but in such small numbers that it was very difficult to find.

Acknowledgments

The leafhoppers mentioned in this paper were identified by Mr. R. H. Beamer of Kansas; the parasites, by Mr. G. S. Walley, and Mr. A. B. Gahan.

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STUDIES OF TOTAL ERYTHROCYTE AND LEUCOCYTE COUNTS OF FOWLS

IV. ERYTHROCYTE AND LEUCOCYTE COUNTS OF BIRDS RAISED IN CONFINEMENT[1]

BY E. I. PALMER[2] AND JACOB BIELY[3]

Abstract

Erythrocyte and leucocyte counts were made of 65 S.C. White Leghorn females and 50 S.C. White Leghorn males which were raised in confinement from the age of one day until sexual maturity.

The mean erythrocyte count of the males was significantly higher than the mean of the females. There was no significant difference between the mean leucocyte counts of the males and the females. The erythrocyte counts of the confined birds were significantly lower than those of normal birds kept under natural conditions and those of one- to two-day-old chicks, while the leucocyte counts were significantly higher.

In a previous paper (1) the authors have reported on the total number of erythrocytes and leucocytes of 100 single-comb White Leghorn adult females and 47 one- to two-day-old single-comb White Leghorn chicks. The mean erythrocyte and leucocyte counts of the adult birds were 2,782,000 ± 22,580 and 32,150 ± 450 respectively; while the mean erythrocyte and leucocyte counts of the chicks were 2,480,000 ± 3176, and 20,600 ± 286 respectively. The difference in the erythrocyte and leucocyte counts of the two groups of birds was in each case statistically significant.

The prevailing tendency to raise young and growing chicks in confinement suggested an investigation of the effects of confinement on the total erythrocyte and leucocyte counts of fowls. While it was originally intended to make bi-weekly counts of chicks from one to two days of age to maturity, the data reported herein are based only on one series of counts.

Material and Methods

The birds used in this investigation consisted of 115 single-comb White Leghorn birds of which 65 were females and 50 males. From one day old to about ten weeks the chicks were raised in battery brooders. They were then transferred to large wire cages where they were kept until they began to lay. The battery brooders and the cages were kept in a large, well ventilated room. No direct sunlight reached the chicks throughout the period of confinement.

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An all-mash ration and water were continually available to the birds. No other feed was given. During and up to the time when the blood counts were made (3½ to 5 months) the birds appeared to be healthy and in excellent condition.

The counts were performed according to the technique reported in a previous paper (4). In the case of several birds, duplicate and triplicate counts were made.

Experimental

Data regarding the total erythrocyte and leucocyte counts of 65 females and 50 males are shown in Tables I and II. It will be seen that the mean erythrocyte count of the males was significantly higher than that of the females. There was no significant difference, however, in the leucocyte counts of the female and male birds. This is in agreement with the results obtained by other investigators. The frequency distribution of the erythrocyte and leucocyte counts is shown in Tables II and III.

TABLE I

STATISTICAL ANALYSIS OF THE ERYTHROCYTE COUNTS OF BIRDS RAISED IN CONFINEMENT, NORMAL ADULT FEMALES AND ONE- TO TWO-DAY-OLD CHICKS

	(1) 65 females kept in confinement	(2) 50 males kept in confinement	(3) 100 normal females	(4) 47 1–2-day-old chicks
Mean erythrocyte count	2164600 ± 24406	254800 ± 25186	2782000 ± 22580	2480000 ± 31576
Standard deviation	291646 ± 17255	264000 ± 17806	334777 ± 15969	318338 ± 22366
Coefficient of variability	13.47 ± 1.79	10.36 ± 0.69	12.03 ± 0.58	12.83 ± 0.91

Difference of the means; Columns 1 and 2: 383400 ± 35071 or 10.93 × P.E.
Columns 1 and 3: 716400 ± 33249 or 18.56 × P.E.

TABLE II

STATISTICAL COMPARISON OF THE LEUCOCYTE COUNTS OF BIRDS RAISED IN CONFINEMENT, NORMAL ADULT FEMALES AND ONE- TO TWO-DAY-OLD CHICKS

—	(1) 65 females kept in confinement	(2) 50 males kept in confinement	(3) 100 normal females	(4) 47 1–2-day-old chicks
Mean leucocyte count	41700 ± 1021	37400 ± 1007	32150 ± 450	20600 ± 286
Standard deviation	12205 ± 722	10558 ± 712	6680 ± 318	2889 ± 202
Coefficient of variability	29.26 ± 1.86	28.23 ± 2.03	20.77 ± 1.029	14.02 ± 0.99

Difference of the means; Columns 1 and 2: 4300 ± 1434 or 2.99 × P.E.
Columns 1 and 3: 9550 ± 1116 or 8.55 × P.E.
Columns 2 and 3: 5250 ± 1103 or 4.76 × P.E.

A statistical comparison of the present data with those obtained in a study of 100 normal adult females kept under natural conditions and 47 one- to two-day-old chicks, reported in a previous investigation (1) is shown in Tables

TABLE III

Frequency distribution of the erythrocyte counts of birds raised in confinement

No. of erythrocytes (000)	Under 1699	1700–1899	1900–2099	2100–2299	2300–2499	2500–2699	2700–2899	2900–3099	3100–3299
% of total birds (65 females)	3.06	4.59	19.89	27.54	29.07	9.18	3.06	1.53	1.53
% of total birds (50 males)				10	24	20	26	18	2

TABLE IV

Frequency distribution of the leucocyte counts of birds raised in confinement

Number of leucocytes	Under 19999	20000–24999	25000–29999	30000–34999	35000–39999	40000–44999	45000–49999	50000–54999	55000–59999	60000–64999	65000–69999	Over 70000
% of total birds (65 females)	1.53	3.06	6.12	18.36	19.89	24.48	6.12	9.18	1.53	4.59	3.06	1.53
% of total birds (50 males)		10	16	24	16	10	10	6	4	4	—	—

I and II. It will be seen that the erythrocyte counts of the 65 females that were raised in confinement were lower than those of the normal adult females or the one- to two-day-old chicks. The difference in the respective means is statistically significant, being over 3.5 times the probable error of the difference. On the other hand, the leucocyte counts of the females and males raised in confinement were significantly higher than those of the normal adult females and the one- to two-day-old chicks. The difference in the respective means is statistically significant.

Discussion

Since the discovery of Vitamin D and the role which it plays in the mineral metabolism of the fowl it is no longer difficult to raise and keep birds in confinement. In fact, with the development of the so-called complete rations and of the laying batteries the whole life span of a chicken can be successfully passed in confinement.

The physiological aspects of the effects of confinement on birds do not appear to have been given due consideration as yet. Recently Buckner *et al.* (2) made some very interesting observations on the subject. They report that White Leghorn cockerels raised in confinement in battery brooders, from which direct sunlight was excluded, developed abnormally large combs and smaller testes than those raised in colony brooders with access to a grass range and receiving direct sunlight. Moreover, recently Jungherr (3) has reported what he considers an important point, that "the absolute and relative lymphatic character of the blood of birds kept under laboratory conditions becomes markedly accentuated." Thus he reports that the average leucocyte count of a group of birds kept under laboratory conditions was 28,816 per cu. mm. when the birds were one month old, 34,962 at two months, and 38,591 at three to four months. Jungherr's data would appear to be in general agreement with the data reported in this investigation.

Much more extensive data than are presented here, however, would have to be gathered before the effects of confinement on total erythrocyte and leucocyte counts could be definitely ascertained. Considering the conditions under which the present series of birds was raised (strict confinement, lack of sunlight, lack of sufficient exercise, etc.), it is not surprising that the erythrocyte and leucocyte counts were affected. The increased number of the leucocytes would tend to indicate that certain factors mitigated against the normal physiological functions of the birds. The increase in leucocytes might be due to (i) mechanical friction resulting from confinement to wire cages; (ii) continual feeding; (iii) incomplete digestion of food; and (iv) bruises. In connection with the latter it may be noted that some of the birds, particularly the males, developed breast blisters. On the whole the leucocyte counts of these birds were not higher, however, than those of birds which did not have breast blisters.

The effect of continuous confinement on the total erythrocyte and leucocyte counts would seem to be of more than passing interest. It is hoped that this preliminary study will stimulate a more comprehensive study of the physiology of confined birds.

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STUDIES ON THE ENDOPARASITIC FAUNA OF TRINIDAD MAMMALS

I. SOME PARASITES OF TRINIDAD DEER[1]

By Thomas W. M. Cameron[2]

Abstract

Four new species of nematodes, referred to three new and one known genera, are described from the deer, *Mazama simplicicornis*, from Trinidad, B.W.I.

While in Trinidad in the early part of 1935, the writer had the opportunity of examining a number of mammals for internal parasites. Owing to lack of time, it was possible to collect only a few species personally, but thanks to the generous co-operation of Professor F. W. Urich of the Imperial College of Tropical Agriculture, a number of animals was secured subsequently and the entrails, after being fixed in formalin, were forwarded to the Institute for dissection and examination. In this way a considerable amount of parasitic material was obtained. The unwanted parts of the carcasses, foetuses, etc., were distributed to other laboratories and museums.

Faunistically, Trinidad is part of the South American Continent, and although local races of animals are recognized in some cases by some mammalologists, there are few, if any, species there which do not also occur in Venezuela. The island, however, is sufficiently far distant from the mainland to prevent the ready exchange of mammals, with the exception of bats, and so the opportunity has been taken to study the parasites of an island community.

Parasites of Deer

A single species of deer—*Mazama simplicicornis*—occurs in Trinidad. This deer was present when the island was discovered by Columbus and, although other species have been introduced to other West Indian islands (such as Tobago and Barbuda) from South America and Europe, there is no history of such an introduction into Trinidad. One is probably justified in assuming, accordingly, that the parasites of these animals have been present since the deer became inhabitants of the island. There are no other ruminants on the island except domesticated sheep, goats, cattle (including zebu) and buffalo of Indian origin. Unfortunately in the short time at my disposal, I was only able to secure a single animal. However, it proved to contain four species of nematode worms of the family Trichostrongylidae, all of them in the small intestine. All appear to be new to science. Fragments of a cestode, closely resembling, if not identical with *Moniezia benedeni* were also seen. In the absence of complete worms however, a more definite identification is impossible.

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Contribution from the Institute of Parasitology, McGill University, Macdonald College, Que., with financial assistance from the National Research Council of Canada.

[2] *Research Professor of Parasitology, McGill University; and Director, Institute of Parasitology, Macdonald College, P.Q., Canada.*

I. *Nematodirus urichi* SP. NOV.

Three females and a single male of this species were recovered. The average length of the females is 18.5 mm., and the length of the male is 10.5 mm.

The cuticle is finely striated both transversely and longitudinally, the transverse striations being about 0.005 mm. apart, while the longitudinal striations are even finer. There are about 20 secondary, more prominent, longitudinal ridges, 0.02 mm. apart: a strip, 0.12 mm. wide on either side of the lateral lines, is free from these ridges. The cuticle on the head of the single male is inflated to form a sphere 0.05 mm. in diameter; the head cuticle of the females, however, is not inflated and is thinner than on the rest of the body. The mouth opening in both sexes is surrounded by a leaf-crown of very minute triangular elements, outside of which lie six circum-oral papillae. Two very small cervical papillae are present about 0.05 mm. from the anterior end.

The oesophagus is of the simple club-shaped type, about 0.6 mm. long in the female. It is 0.075 mm. wide at its maximum diameter but it is slightly swollen at its anterior end. A single large anterior oesophageal tooth is present. (Fig. 1). The nerve ring is situated about the junction of the anterior and middle third and the excretory pore lies at the level of the junction of the oesophagus and intestine.

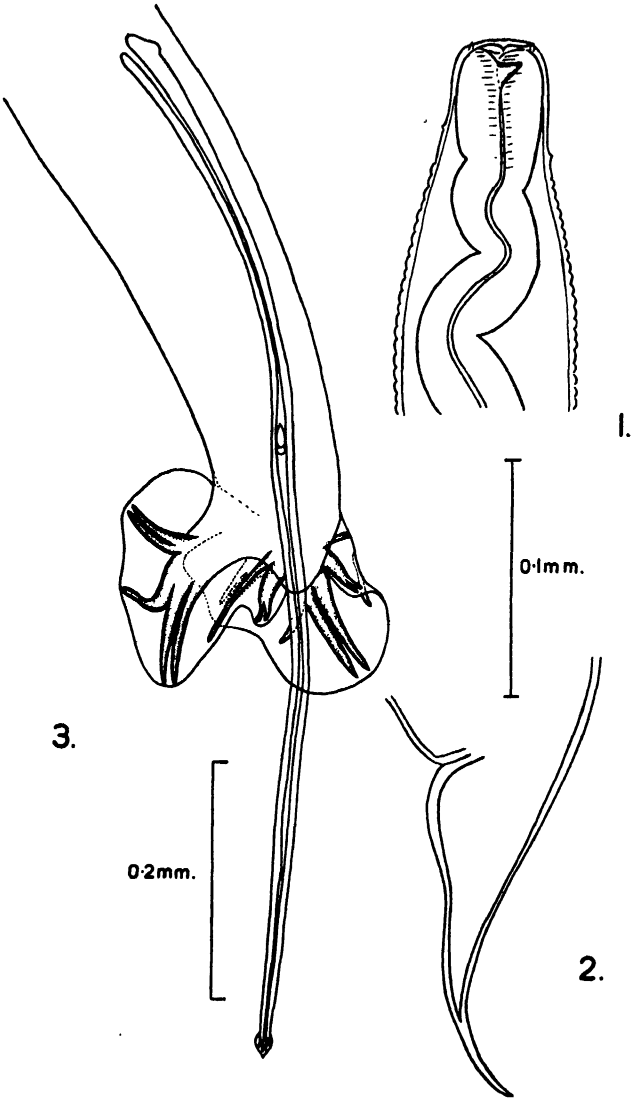
Unlike the species of *Nematodirus* found in domestic ruminants in temperate climates, the tail of the *female* is *not* truncated but is prolonged into a conical tip. Anterior to the anus, which is 0.125 to 0.15 mm. from the tip of the tail, (Fig. 2) the width of the body quickly increases to a diameter of 0.2 mm.

Both genital tubes originate in the anterior part of the body but the posterior ovary passes backwards to just in front of the anus. There it turns on itself and almost immediately becomes transformed into uterus. The two uteri are consequently opposed to each other. The genital tubes make several coils round the intestine. The ovejector is of the usual type with each arm 0.5 mm. long with a central "bulb" and a short common vagina which opens about 8 mm. from the tail—just posterior to the middle of the body. The eggs are of the large *Nematodirus* type, with a relatively thick shell, and measure 0.15 mm. by 0.08 mm. They have reached the eight-cell in the ovejectors.

The male has a typical *Nematodirus* type of bursa (Fig. 3) with two large lateral lobes. The dorsal lobe is split into two parts, each being fused with the corresponding lateral lobe.

The dorsal ray is also completely split, each element ending in two digitations. The externo-dorsal ray is long and slender, running parallel with, but quite separate from the postero- and medio-lateral rays and terminating about midway between their tips and the junction of the dorsal and lateral lobes. The postero- and medio-lateral rays are parallel to each other. The ventral rays are also parallel with each other and are situated at right angles to these two lateral rays. The externo-lateral lies almost mid-way between these two pairs but, unlike them it does not quite reach the edge of the bursal membrane. The central part of the medial surface of each lateral lobe of the bursa is covered with numerous oval swellings.

The spicules are long and filiform, measuring 0.9 mm. in length. They are fused at their tip to form a heart-shaped termination, which is constricted at the base and in which each spicule bifurcates. A very small, transparent gubernaculum is present. Caudal papillae are absent.



Nematodirus urichi SP. NOV.

FIG. 1. *Head of female.* FIG. 2. *Tail of female.* FIG. 3. *Tail of male.*

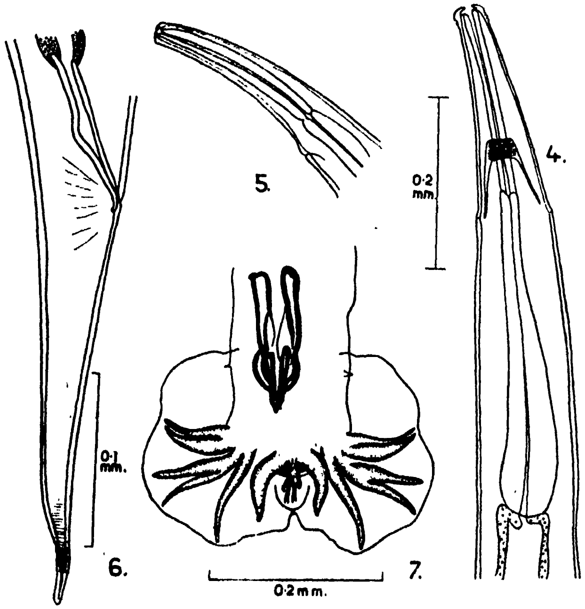
This species differs in several important respects from other members of the genus, notably in the head region and the tail of the female. The name *Nematodirus urichi* sp. nov. is accordingly proposed for it. It may be distinguished from other known species by the leaf-crown of minute elements and by the pointed tail of the female.

II. *Mazamastrongylus trinitatis* GEN. ET SP. NOV.

Several females and two males of this species were obtained. The average length of the females was 7.7 mm. and of the males, 6 mm.

No transverse striations are visible on the cuticle except on the head of both sexes and on the tail of the female. There are, however, about 20 prominent longitudinal striations which are not quite continuous and somewhat variable in number. Between these are numerous very fine longitudinal striations.

The cuticle of the head region is not inflated but the actual anterior end is distinctly dome-like with a central mouth opening surrounded by six small circum-oral papillae. Immediately behind this are some half-dozen rather coarse transverse striations, which are more prominent in the female than in the male. (Figs. 4 and 5). The oesophagus is divided into two distinct parts. The anterior part is 0.2 mm. in length and 0.025 mm. in width while the posterior part is 0.4 mm. long with a maximum width of 0.075 mm. The dimensions are similar for both sexes.



Mazamastrongylus trinitatis GEN. ET SP. NOV.

FIG. 4. *Head of female (dorsal view).* FIG. 5. *Head of male (lateral view).*
FIG. 6. *Tail of female.* FIG. 7. *Tail of male.*

Two small cervical papillae are present at the level of the junction between the two parts of the oesophagus. The nerve ring surrounds the posterior portion of the tubular anterior part. The excretory pore is prominent and is situated just posterior to the cervical papillae.

The female has a long, somewhat bluntly pointed tail, 0.24 mm. in length. (Fig. 6.) Just anterior to the tip, which is covered with minute spines, there is a number of somewhat coarse transverse striations; these become gradually finer, to disappear completely about a quarter of the distance from the anus. Anterior to the anus, the body rapidly increases to its maximum width of 0.16 mm.

Both genital tubes originate in the middle region of the body. The anterior tube passes forward, turns about 2 mm. from the head, and passes directly backwards. The posterior tube passes backwards almost to the anus when it turns forward again. Both tubes are slightly undulating but neither actually encircles the intestine. The two uteri are opposed and relatively long. As a rule a single row of eggs lies in them with their longitudinal axes across the body, but occasionally two eggs lie abreast in the other direction. The ovejectors are short but massive and are divided into two *pars muscularis*, each 0.15 to 0.2 mm. long and a common, very thick *pars ejectrix* 0.2 mm. long. The vagina is very short and the vulva is an unprotected slit about 1.5 mm. from the tail, dividing the body in the ratio of 4 : 1.

The eggs are thin-shelled and oval and measure 0.07 mm. by 0.05 mm. They had reached the morula stage at the end of the uteri.

The male bursa has two large lateral lobes and a small dorsal lobe (Fig. 7). The dorsal ray is short and stout and bifurcated for about half its length. Each bifurcation carries three digitations, the two medial being long and pointed while the other is shorter. The externo-dorsal ray is stout and curved. It is considerably shorter than the postero-lateral ray, which is separated from the other two lateral rays. The medio- and externo-lateral rays lie close together but their tips diverge, the externo-lateral being the stouter. The ventral rays are relatively large and are separated from the lateral group. They are directed away from them and are parallel to each other throughout their entire length.

An incipient dorsal lobe, supported by two weak curved rays, is present within the main bursa, anterior to and ventral of the main dorsal lobe.

Small pre-bursal papillae are present at the junction of bursa and body.

The spicules are similar, short and irregular in outline. They measure about 0.16 mm. long. They have four points. Ventrally there is a blunt club-shaped termination directed somewhat laterally. In the medial plane there are two sharp points, one straight and one undulating. In both specimens, the undulating points of both spicules were in close apposition to give a spear-shaped tip. Dorsally there is a shortish, blunt point ending in a definite, but irregular knob. No accessory pieces could be seen.

The seminal vesicle extends for about half the body length. The testicular tube is almost straight and originates about 0.75 mm. posterior to the mouth opening.

This parasite shows affinities to both *Ostertagia* and to *Cooperia* but differs from both in several important respects. Notably, it differs in the division of the oesophagus into two parts. The writer has been unable to assign it to any known genus and accordingly the new name *Mazamastrongylus trinitatis* gen. et sp. nov. is proposed for it. It may be distinguished by the division of the oesophagus into two parts, presence of cervical and pre-bursal papillae and of an accessory bursal membrane, and its *Cooperia*-like head.

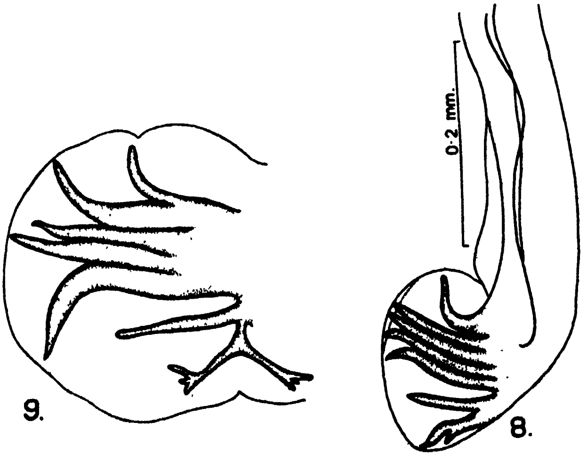
III. *Ierestrongylus filiformis* GEN. ET SP. NOV.

Three males but no females of this species were found, in a poor state of preservation. They have an average length of 6.5 mm.

The cuticle is finely striated transversely with about 20 longitudinal striations as well. There is a conspicuous ventral crest present which runs from just behind the head to the origin of the bursa.

In two of the specimens the cephalic cuticle is distinctly inflated; in the third it has a terminal cap closely resembling that of *Cooperia*. The mouth opening is simple and surrounded by six small papillae. The oesophagus is of the simple claviform type, 0.35 mm. long. Cervical papillae are small and situated about half way down its length. The excretory pore is situated just anterior to the oesophageal-intestinal junction.

The bursa (Figs. 8 and 9) is relatively voluminous with two large lateral lobes separated from each other by a small dorsal cleft; there is no dorsal lobe. The edge of the bursa is "scalloped" and there are numerous small projections on its inner surface.



Ierestrongylus filiformis GEN. ET SP. NOV.
FIG. 8. *Tail of male (lateral aspect).* FIG. 9. *Diagram of bursa (ventral view).*

The dorsal ray is divided throughout most of its length, one part lying in each lateral lobe. Each bifurcation terminates in three digitations, the medial of which is very small and the ventral recurved.

The externo-dorsal ray is relatively stout and lies midway between the lateral group and the dorsal ray. It does not reach the edge of the bursa. The three lateral rays and the latero-ventral are about the same size and lie parallel with each other for most of their length. The outer members of the group however, curve outwards at their tips whereas the two central ones are more or less straight. The ventro-ventral ray is smaller than the others and curved anteriorly.

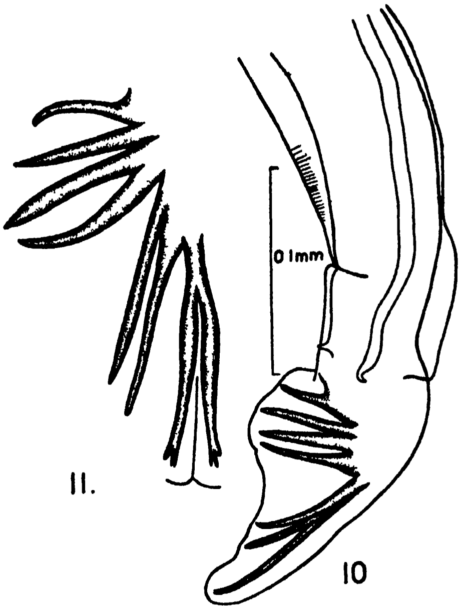
No pre-bursal papillae were seen. The spicules are equal, long and filiform, measuring 0.65 mm. in length. The tip of each is bifid. No accessory pieces were observed.

It is realized that it is unsatisfactory to describe a new species on the basis of males alone, but these males differ so distinctly from any previously recorded from ruminants, that it seems desirable to put their description on record. Unfortunately, without females, it is impossible to refer them to their proper sub-family although their appearance suggests that they belong to the Heligmosominae rather than to the Trichostrongylinae. The bursal formula somewhat recalls that of the genus *Trichostrongylus*, but it differs in the long filiform spicules, the absence of a gubernaculum, and the presence of a ventral crest. These points, even in the absence of the female, seem to justify the creation of a new genus and the name *Ierestrongylus filiformis* gen. et sp. nov. is proposed for it.

IV. *Mazamanema longibursatum* GEN. ET SP. NOV.

A single male, in a poor state of preservation, was obtained. It measures 2.3 mm. in length with a maximum breadth of 0.075 mm. It was lying in a flat spiral when collected but was easily extended. The cuticle is finely striated transversely and has about 14 faint longitudinal ridges and a small ventral crest. The head is simple and rounded with six inconspicuous circum-oral papillae. There are two obvious cervical papillae, 0.05 mm. from the anterior end. The oesophagus is simple and club-shaped, nearly 0.3 mm. in length.

The bursa is long and without a dorsal lobe. (Fig. 10). There is, however, a deep dorsal cleft between the two lateral lobes. The dorsal ray is very long (Fig. 11) and split for nearly its entire length, each bifurcation terminating in three small digitations. The main arms of this ray are almost parallel with each other throughout their entire length. The externo-dorsal ray is long and thin and diverges only slightly from the dorsal ray. The postero-lateral ray is similar and is situated in close proximity to the externo-dorsal and widely separated from the other lateral rays. These, together with the two ventral rays, are situated in a group at right angles to the main axis of the body. The two laterals and the latero-ventral are thin and somewhat elongated, whereas the ventro-ventral is considerably shorter. Small pre-bursal papillae are present just in front of the origin of the bursa and just in front of them is a distinct ventral groove at the termination of the ventral crest. The spicules are equal, long and filiform, each measuring 0.22 mm. Each ends in a single point, which is somewhat hook-shaped. No accessory pieces were seen.



Mazamanema longibursatum GEN. ET SP. NOV.
FIG. 10. *Tail of male (lateral aspect).* FIG. 11. *Diagram of bursa (ventral view).*

The remarks on the preceding species apply even more strongly to this form. The male differs, however, from all known members of the family by the possession of a ventral crest, a pre-bursal groove, long slender papillae and by the bursal formula which is characterized by the elongated split dorsal ray, with the externo-dorsal and postero-lateral close to it, and by the grouping of the other four rays distant from them.

The name *Mazamanema longibursatum* gen. et sp. nov. is accordingly proposed for it.

Acknowledgment

I have to acknowledge the ready help and assistance I received from interested persons in Trinidad. Especially must I acknowledge the assistance of Mr. H. V. Metivier, B.Sc., M.R.C.V.S., the Government Veterinary Surgeon, who generously placed his laboratory at my disposal.

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GROWTH OF *MYA ARENARIA* L. IN THE BAY OF FUNDY REGION[1]

BY CURTIS L. NEWCOMBE[2]

Abstract

Data are presented which demonstrate the validity of "annual rings" as age determinants in *Mya arenaria* L. The time curve of growth for this latitude depicts a relatively rapid rate during the first four years, after which a pronounced decrease obtains. Relatively little variation has been found in the rate of growth characterizing the widely separated parts of the Bay studied. In general, specimens 18 mm. in length grow about 12 mm. (66%) in one year and those 26 mm. grow about 9 mm. (33%). These represent typical values for growth in the Bay of Fundy region under favorable conditions.

The length of the growing season during 1931 was approximately four months, —May to August, inclusive. During 1930, growth continued until about Dec. 1. Such a late fall growth is considered unusual for the Bay of Fundy region.

Data are presented which show that variations in seasonal growth rates during the same and different years correspond with abundance of diatoms and not with temperature. The rate of growth has been found to vary directly with time of submergence. Wide variations in salinity and sulphide content of the beach are required to reduce the amount of growth significantly. Excess surface silt on the beach limits the growth rate and survival time of *Mya arenaria.*

I. Introduction

Comparatively few growth studies of marine pelecypods have been conducted under natural conditions. Mead and Barnes (14), Kellogg (9) and Belding (1) are among the early contributors to our knowledge of the growth of *Mya arenaria* on the east coast of the United States. In view of the lack of information dealing with the growth of this species in the colder and more northern waters of the Bay of Fundy, the present study was undertaken.

The purpose of the investigation has been; first, to determine the validity of periodic rings on the valves for age determination; second, to ascertain the normal rate of growth that is representative of the latitude in question; and third, to evaluate the influence of certain environmental factors on distribution, growth rate and survival by using rate of growth in nature as a criterion. Certain ecological aspects of the problem have received special consideration.

In order to confirm and enlarge the data obtained in the study under natural conditions, experimental boxes were planted on various beaches at different tidal levels, and the specimens placed therein were measured at particular intervals to determine the seasonal and annual rates of growth. In this way, it has been possible to establish the significance of the concentric rings on the shells for age determination. The two principal length classes of *Mya*

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Contribution from the Zoological Laboratories of the University of Toronto, the Chesapeake Biological Laboratory and the University of Maryland; with financial assistance from the National Research Council of Canada.

[2] *Ecologist, Chesapeake Biological Laboratory, and Instructor in Zoology, University of Maryland, College Park, Md., U.S.A.*

arenaria employed are designated "small" and "large" clams. As a consequence of the correspondence obtained between relative growth rate and abundance of plankton organisms present, particular attention has been given to the composition of the food of this lamellibranch.

II. Experimental Methods

The linear dimensions of *Mya arenaria* were measured with a vernier caliper reading to 0.1 mm. The length measurement is the greatest anterior-posterior dimension and the width constitutes the greatest radius with the umbo as a centre. In view of the shape of the valves, this width dimension was accepted rather than a measurement made on a line at right angles to the long axis (Fig. 2, A). Throughout the paper, length is used as a basic index of size. Other indices such as width, thickness, weight of shell and dry weight of body parts have been studied and will be treated in a subsequent paper.

Measurements of "annual rings" for determining age were made with dividers which were transferred to a vernier caliper and read in millimetres, tenths being estimated. On account of the width of the groove constituting the winter check, a vernier measurement does not possess greater accuracy.

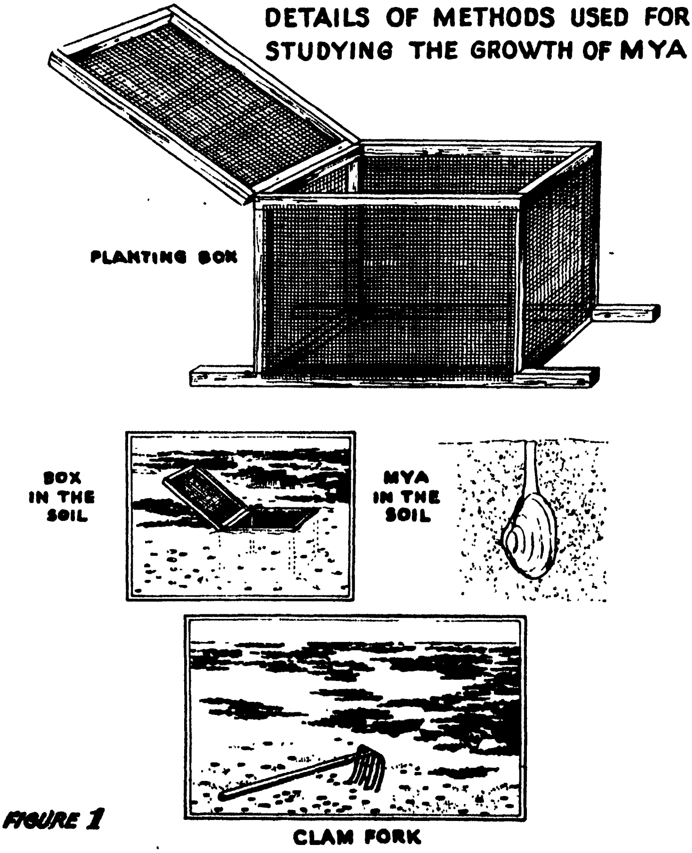

FIG. 1. *Showing materials of Mya experiments.*

Increments of growth in nature under different experimental conditions were determined by planting specimens in experimental boxes placed in the desired natural habitat. The boxes consisted of a solid wooden frame covered with a wire screening of 5 mm. mesh, their dimensions being 30 × 30 × 20 cm. (Fig. 1). The soil used in the boxes was obtained from the environments under consideration.

Groups of 45 clams were planted in each box. Usually, these were measured at five times: (i) the beginning of the experiment, February 28 to March 4, 1931, (ii) June 18 to 22 for obtaining "spring" growth, (iii) August 16 to 20 for "summer" growth, (iv) October 12 to 16 for "fall" growth, and (v) December 2 to 5 for "late fall" growth. During 1932, records were taken for the corresponding "spring" and "summer" periods.

Specimens were selected for planting so that the range of length within each group (45) was as small as possible (seldom exceeding 3 mm.) with a view to reducing the variability. In several experiments, designed to compare the effect of environmental conditions on rate of growth, specimens of the same length (pairs) were planted in the areas under comparison; therefore, at the beginning of the experiment, the groups possessed the same mean lengths and other mathematical constants. The advantage of "pairing" is to reduce the variability between the groups in order that the mean difference, if any, may be interpreted as due to environmental factors and not to variability between the groups.*

In all mathematical calculations, values have been computed to at least three places beyond the decimal, although recorded only to the nearest first or second place, depending on accuracy demanded.

III. Growth in Nature

Four beaches were selected for the study of growth under natural conditions. These are located at St. Andrews, New Brunswick; Economy Point, Nova Scotia; Clam Cove, New Brunswick; and Sissiboo River, Nova Scotia.

Opinion concerning the value of periodic rings for estimating the age of bivalve molluscs has not been unanimous. Mead and Barnes (14) question the validity of periodic rings on the valves of *Mya arenaria* for age determination. A study of the Bay of Fundy specimens, the valves of which differ significantly from those of southern regions, has indicated that certain rings are formed annually and constitute a valid basis for age determination. This validity is not the same throughout life.

In clams over four years of age, it is difficult to discern with assurance the first ring and frequently this also applies to the second. To obtain values representing the increment of growth during the first two or three years, large numbers of specimens, one, two, and three years of age were measured, since in young specimens these rings are more easily recognized. Cognizance must be taken of the fact that whereas mean values, obtained by measuring rings of a heterogeneous population ranging in age from three to twelve

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**Standard error has been used throughout.*

years, represent average growth increments, the result of average climatic conditions, those obtained by measuring a group of the same age imply a constant climatic factor. For this reason, the data obtained in these two ways are not directly comparable. To ascertain the extent of variation that exists, mean length values were obtained for the third "annual ring" (a) from a heterogeneous population varying in age, and (b) from a population all of which were in their fourth year of growth, namely, 1932. The length of the third ring was, in the case of each specimen, a result of the same climatic conditions, *i.e.*, those of 1931. No very significant difference was indicated, the variation not exceeding 1.5 mm.

TABLE I

CONSTANTS OF FREQUENCY DISTRIBUTIONS OF LENGTHS OF ESTIMATED ANNUAL RINGS OF *Mya arenaria* L. IN THE ST. ANDREWS REGION

No. of specimens	Ring no.	Mean length in mm.	Standard deviation	C.V.
	1			
185	2	21.6±0.4	5.68±0.29	26.3±1.4
178	3	32.0±0.1	0.83±0.04	2.6±0.1
173	4	42.0±0.1	1.59±0.08	3.8±0.2
150	5	51.7±0.2	2.60±0.15	5.0±0.3
108	6	59.4±0.4	3.80±0.26	6.4±0.4
100	7	65.3±0.3	2.99±0.21	4.6±0.3

In the beginning, some other difficulties may be experienced in recognizing the probable annual ring as distinct from certain less prominent rings formed throughout the year, and in some cases, from those due to mechanical agencies (*cf.* 14, p. 55). The consistency of the shell is such that dark rings are often

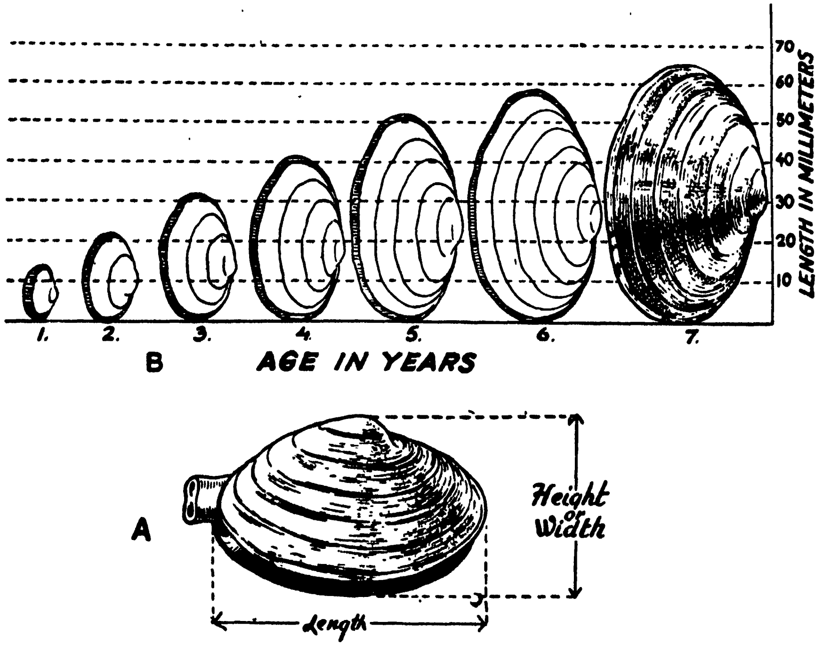

FIG. 2. A. *Outline of Mya arenaria showing "annual rings" and the dimensions measured.* B. *Estimated lengths of Mya arenaria at different ages as shown by annual-ring measurements of the St. Andrews collection.*

formed when the individual is disturbed. An example of this phenomenon is provided in Fig. 6, which shows the two faint lines produced when clams were taken up for measurement in June and August. Usually, however, the annual ring is wider, and more consistent and regular than the less prominent rings (Fig. 2).

On the basis of observations of specimens ranging in size from 13 mm. to 65 mm., grown in planting boxes, it appears that the mode of formation of disturbance and winter rings is quite similar (*cf.* 19). In the case of very old specimens* rings after the eighth or ninth are frequently indistinct, the annual increment of growth being small.

Collections were taken at approximately the same level on each beach, namely, eight feet above chart datum. For the purpose of comparing growth in nature at different levels on the same beach, an additional collection was made in Clam Cove at a level of sixteen feet.

1. St. Andrews, N.B.

Specimens ranging in age from four to twelve years were collected for ring measurement from this beach in October, 1931. On account of the wearing away of the shell in the umbo region, a satisfactory value was not obtained for the length of the first ring. It is seen from Table II that the absolute yearly increment of length is quite similar for the third, fourth, and fifth years, namely, about 10 mm. The decrease during the sixth and seventh years is very marked (Figs. 2 and 3). The total lengths and widths of estimated annual rings have been plotted against age and are shown in Figs. 2 and 3.

TABLE II

Yearly increments of growth of *Mya arenaria* as indicated by estimated annual-ring measurements in the St. Andrews region

No. of specimens	Ring no.	Mean length in mm.	Absolute yearly increment of length, mm.	Yearly percentage additions of length
	1			
185	2	21.6		
178	3	32.0	10.3	47.8
173	4	42.0	10.1	31.5
150	5	51.7	9.7	23.0
108	6	59.4	7.6	14.8
100	7	65.3	5.9	9.9

An expression of the yearly percentage additions of length, as shown in Table II, gives a more accurate idea of the decreasing growth increment with increasing age (Fig. 3). In this figure the initial length at the beginning of each year is given first, and immediately above is the absolute annual increment and then separately and higher up the corresponding percentage additions are represented.

**The largest clam examined by the writer in which the age was quite definitely indicated by the rings was in its seventeenth year, the total length being 98.4 mm. Starting with the second, the lengths of the respective rings are as follows: Number II—13.7 mm., III—28.3 mm., IV—47.5 mm., V—56 mm., VI—63.2 mm., VII—71.3 mm., VIII—78 mm., IX—82 mm., X—85.2 mm., XI—88 mm., XII—90.5 mm., XIII—92.8 mm., XIV—94.5 mm., XV—96.8 mm., XVI—98.4 mm.*

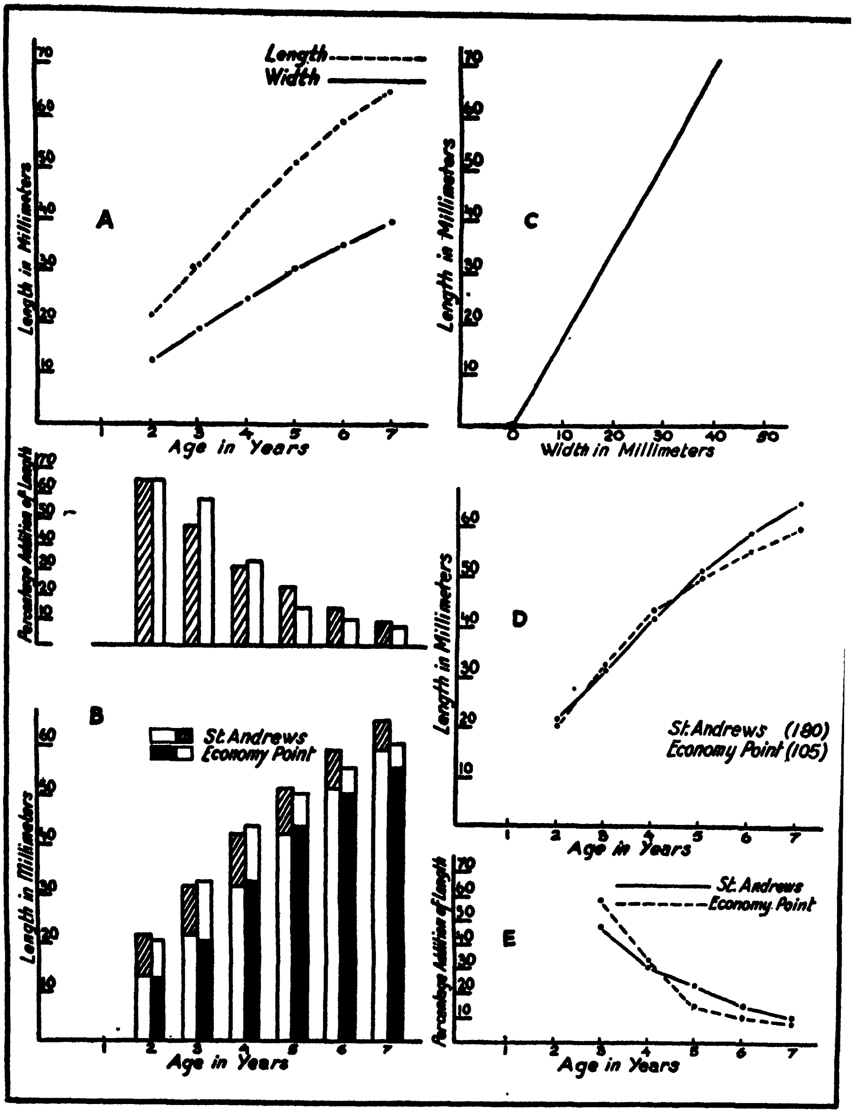

FIG. 3. A. *Total lengths and widths of estimated annual rings plotted against age. This figure is based on mean values presented in Table I.* B. *Yearly increments of length of Mya arenaria as indicated by annual rings on valves. Length of first ring not definitely known.* C. *Length-width relation.* D *to* E. *Absolute yearly growth increments of Mya as shown by annual rings.* D. *Absolute growth;* E. *Percentage additions of length.*

2. ECONOMY POINT, N.S.

The rate of growth of clams at the head of the Bay of Fundy is very little different from that of the mouth region as indicated by a study of the specimens collected at Economy Point (Tables III and IV). The mean lengths at the end of the second, third, fourth and fifth years are quite similar to the lengths of the corresponding year rings of the St. Andrews collection (Table I). The yearly percentage additions in length for both collections of clams are given in graphic form (Fig. 3).

TABLE III

CONSTANTS OF FREQUENCY DISTRIBUTIONS OF LENGTHS OF ESTIMATED ANNUAL RINGS OF *Mya arenaria* L. IN THE ECONOMY POINT REGION

No. of specimens	Ring no.	Mean length in mm.	Standard deviation	C.V.
	1			
105	2	20.7±0.2	1.89±0.13	9.1±0.6
103	3	32.8±0.3	3.64±0.25	11.1±0.8
105	4	43.8±0.4	3.90±0.26	8.9±0.6
100	5	50.5±0.4	3.99±0.28	7.9±0.6
99	6	55.9±0.5	5.41±0.38	9.7±0.6
61	7	60.1±0.6	4.57±0.41	7.6±0.6

TABLE IV

YEARLY INCREMENTS OF GROWTH OF *Mya arenaria* AS INDICATED BY ESTIMATED ANNUAL-RING MEASUREMENTS IN THE ECONOMY POINT REGION

No. of specimens	Ring no.	Mean length in mm.	Absolute yearly increment of length, mm.	Yearly percentage addition of length
	1			
105	2	20.7		
103	3	32.8	12.1	58.4
105	4	43.8	11.0	33.7
100	5	50.5	6.7	15.2
99	6	55.9	5.4	10.7
61	7	60.1	4.2	7.5

3. CLAM COVE, N.B.

Specimens were collected from two levels of the beach at Clam Cove in August, 1932, for the purpose of comparing rate of growth in nature as indicated by "annual ring" measurements with that in experimental planting boxes (see page 111). The collections were made at levels of 8 ft. and 16 ft. above chart datum.

It was not possible to secure a reliable mean value for the first ring from the specimens of either collection on account of the shell being worn away in the umbo region. The value, 3.9 mm., for the low level was obtained by measuring the single ring on specimens collected in May, 1932, and representing 1931 set. The figures in Table V showing the amount of growth during the first summer (1931) are not directly comparable with the remaining data and are included only for the reader's convenience.

The average mean lengths of the rings of clams collected at the 16-ft. level are significantly less than those of the specimens from the low level (Table V). At the upper level, it is apparent that the absolute yearly increments of growth are approximately 50% of the corresponding increments for the less exposed level. At the eight-foot level the percentage addition in length during the third year is 87.0 as compared with 72.7 for the high level. The percentage additions for the succeeding years decrease markedly at both levels and, it is noted, in a fairly similar fashion (Table V).

**In the text, values for ring number one are, for convenience, referred to as though growth over a period of one year was implied. In reality, the period is less than four months, since it is not probable that set occurs in any abundance before August 1.*

The apparent 50% reduction in rate of growth at the more exposed zone of the beach is shown by a comparison of the growth rates produced at the two levels by clams possessing similar initial lengths at both levels, namely, 15.8 mm. and 28.1 mm. at the upper, and 15.7 mm. and 29.3 mm. at the lower level. Cognizance must be taken of the difference in age when making a comparison. These results are of particular interest on account of the similar retardation accompanying increase of exposure illustrated by field experiments (page 118). Thus, at the upper level, it may require nearly six years for a clam to reach a size ordinarily attained in three years at the lower and less exposed areas.

A comparison of the corresponding mean values obtained in 1931 for clams growing in the immediate vicinity of St. Andrews (Table I), with the Clam Cove results (1932) reveals a very close correspondence. With the exception of the value for the second ring, the difference usually does not exceed 2 mm.

The value representing the mean length at the end of the second year in the Clam Cove area is 15.7 mm. whereas that for the St. Andrews region is 21.6 mm. In order to check the accuracy of the former value, a separate group of one hundred clams, all of which were 1929 set, were measured, and the value thus obtained for the second ring was 16.4 mm., and for the third, 30.4 mm. These results confirm those for the second and third rings presented in Table V and imply that the difference is significant.*

TABLE V

YEARLY INCREMENTS OF GROWTH OF *Mya arenaria* AS INDICATED BY ESTIMATED ANNUAL-RING MEASUREMENTS. (CLAM COVE REGION)

No. of specimens	Ring no.	Mean length in mm.	Absolute yearly increment of length, mm.	Yearly percentage additions of length
Level: 16 feet above chart datum				
	1			
172	2	9.1		
152	3	15.8	6.6	72.7
125	4	22.5	6.7	42.3
49	5	28.1	5.6	25.1
28	6	32.7	4.5	16.2
16	7	36.1	3.4	10.5
Level: 8 feet above chart datum				
70	1	3.9		
242	2	15.7	11.8	301.8
245	3	29.3	13.6	87.0
148	4	40.5	11.2	38.3
67	5	48.5	8.0	19.8
57	6	57.5	8.9	18.4
39	7	63.8	6.3	11.0
22	8	69.4	5,6	8.8

A similar result has been obtained by Fraser (6) at Dingle Beach on Mersey Estuary, near Liverpool, England, who states that specimens apparently showing two summers' growth average 1.7 cm. in February. (Level about 20 ft., chart datum being 4 ft.)

4. SISSIBOO RIVER, N.S.

Specimens for "annual ring" measurements were collected at an 8-ft. level on the Sissiboo beach in July, 1932. The mean values obtained correspond quite closely to those of the above-mentioned regions (Table VI). The data for all rings except number one were obtained from a single collection composed of clams of various ages, therefore the results are not likely to be appreciably influenced by yearly variations in climatic conditions. The value for ring number one, 4.9 mm., represents the average amount of growth in the case of clams which spawned in 1931, and was obtained by measuring a group of one hundred clams collected on July 14, 1932. In addition to measurement of the single ring present on specimens of this collection, records of total length were also made, the mean value being 14.5 mm. This represents an absolute increment of 9.6 mm. (195.9%) during approximately the first half of the growing season of 1932. The absolute increment obtained for the second year of growth is 15.4 mm., hence considerably more than one-half (9.6 mm.) of the growth during the second year had occurred prior to July 14. These results, signifying a relatively rapid growth rate in May and June, are quite similar to those obtained in the Passamaquoddy Bay region during 1931.

TABLE VI

YEARLY INCREMENTS OF GROWTH OF *Mya arenaria* AS INDICATED BY ESTIMATED ANNUAL-RING MEASUREMENTS IN THE SISSIBOO AREA

(Level; 8 feet above chart datum)

No. of specimens	Ring no.	Mean length in mm.	Absolute yearly increment of length, mm.	Yearly percentage addition of length
100	1	4.9		
120	2	20.2	15.4	315.8
84	3	31.0	10.8	53.4
28	4	40.3	9.2	29.8
19	5	48.7	8.4	20.8
11	6	55.2	6.5	13.4

The percentage addition of length during the second year was very large, namely, 315.8; for the third year it was 53.4 and during succeeding years it gradually decreased until in the sixth year it was only 13.4. The mean value for the second ring, 20.2 mm., is significantly larger than that for the Clam Cove collection, whereas the mean length of the third ring is essentially the same in both groups (Tables V and VI). To check the accuracy of the lengths of the second and third rings of the Sissiboo collection, a separate group obtained from a similar level, all 80 specimens of which represented the 1929 set, was measured, and the mean length values calculated. The results obtained are as follows:—ring No. 2, 19.7 mm.; No. 3, 29.7 mm. The small differences obtained, of slightly over one mm., are obviously not significant, and constitute a reliable check of the accuracy of the values for the second and third rings. The absolute increments for the third, fourth and fifth years are essentially the same, namely, about 9.0 mm. and the corresponding increment for the sixth year is 6.5 mm.

To ascertain the differential growth rates occurring in very small clams growing under natural conditions, two groups, each of which contained thirty specimens in their second year of growth (1932), were selected by the method of random sampling. In one group the length of the single annual ring present ranged from 3.0 to 3.9 mm.; in the remaining group the corresponding range was 6.0 to 6.9 mm. The respective mean values obtained are 3.4 mm. and 6.2 mm.; these values will hereafter be referred to as the initial lengths of the respective groups. By measuring the total lengths, it has been possible to determine the increment of growth this year (1932) up to the date of collection (July 14) for specimens possessing initial lengths of 3.4 mm. and 6.2 mm. The average absolute increment for the smaller group was 9.0 mm. (264.7%). These values for the larger group were 9.8 mm. and 158.1%. It is shown that a marked decrease in the percentage increment occurs with increase in size, which fact has been previously suggested in Tables V and VI.

Table VII has been prepared by averaging the results of ring measurements of the above-mentioned four collections (St. Andrews, Economy Point, Clam Cove and Sissiboo River). In general, the agreement of the results obtained from the various collections is sufficient to indicate that the averages presented give a fairly accurate picture of the growth phenomena characteristic of the Bay of Fundy Region (*cf.* 29, 30 and 31).

TABLE VII

AVERAGE YEARLY INCREMENTS OF GROWTH OF *Mya arenaria* IN THE BAY OF FUNDY AS SHOWN BY ANNUAL-RING MEASUREMENTS

Ring no.	Mean length in mm.	Absolute yearly increment of length, mm.	Yearly percentage addition of length
1	4.4		
2	19.5	15.2	346.3
3	31.3	11.7	59.9
4	41.7	10.4	33.3
5	49.8	8.2	19.6
6	56.9	7.1	14.3
7	63.0	6.1	10.7

IV. Growth Experiments Under Natural Conditions

Attention has been given, firstly, to certain more important factors operating at the Clam Cove beach under what may for convenience be termed *normal conditions*, together with the growth rate characterizing these conditions; and secondly, to those factors which retard the growth processes as revealed by growth experiments set up in environments suitable for factorial analysis. The selection of these environments is beset with problems. Notwithstanding this fact, it is felt that certain positive evidence has accrued.

1. NORMAL CONDITIONS

Primary factors of the environment which are considered significant in the light of their effect on growth are period of submergence, circulation of water, food, salinity, temperature and soil. The beach at Clam Cove was studied for the purpose of evaluating certain of the above factors from the standpoint of growth.

(a) Description of a Typical Beach—Clam Cove, N.B.

The beach at Clam Cove is located on the east side of Deer Island near Fairhaven, N.B. The contour of the shore serves as a natural protection against heavy north and west winds as well as the excessive tidal action so strong in the nearby strait.

Size and slope. The area of this beach is approximately 55 acres, of which at least 30 acres are suitable for commercial digging. The slope is very gradual and quite uniform, there being a progressive diminution in the direction of the water. An idea concerning the degree of slope may be given by the fact that the distance between mean high-water and mean low-water level is about 400 metres and the average width nearly 200 metres.

Soil. The soil consists of a mixture of sand and mud, the proportions varying at different vertical levels. Above and near the high tide level sand predominates, and there are indications of some shifting. At a distance of about 50 metres below the mean high-water level, a high percentage of mud is present, the proportion increasing steadily at the lower levels. Below the mean low-water level there is a thin surface layer of soft mud which exerts a deleterious effect on growth. The soil is everywhere deep and in general unaffected by decomposing materials. At the lower levels, shallow tide-pools are present and an occasional seepage stream may be observed. Except at the very low levels, it is evident that the edaphic features of the environment are very favorable for the growth of *Mya arenaria.*

Salinity. The salinity records, although irregularly taken, indicate that the variation in salt content of the water at the head and at the mouth of the cove is slight at flood tide and that salinity changes take place in the soil water during exposure. In a paper on the physical factors of the sand beach (15) it is shown that the changes of salinity of soil water, caused by evaporation and subsurface seepage during exposure, are very slight. A change of 0.2% may occur in a large shallow tide-pool exposed for several hours.

Food. The excessive mixing of water in the Passamaquoddy region may be considered favorable for the production of food in this area. Unfortunately during the first two years of the investigation (1930-1931) the author was unable to obtain concrete data pertaining to the food of *Mya.* Indirect information on the food (diatoms) during the above period may be obtained from the studies of Davidson (3) regarding the abundance throughout 1931. These studies indicate that the diatoms begin to increase in March, reach their maximum in May or June, and then decline with, in some years, a less rapid decline in the autumn, usually in September or October. In Fig. 8 there is shown a logarithmic curve of diatom abundance during the period of the growth experiment.* It is of immediate interest to note that the diatom abundance during the fall and late fall periods of 1931 was very small, being significantly less than that of the previous year. Doctor Davidson states that the figures for August, 1931, are principally due to the dominance of *Chaetoceros socialis* which started on August 5 and continued until September.

**Personal communication from Dr. V. M. Davidson.*

In 1930, *Chaetoceros socialis* (4–15 μ) was also abundant from August 20 to September 15, although at this time a larger-celled species, *Chaetoceros debilis*, (12–29 μ) was also present.

During the summer of 1932, an attempt was made to determine directly the more common organisms which compose the food of *Mya*. On account of the small size of the stomach and the difficulty of isolating all of the fluid contents, it has not been possible to secure exact quantitative data. The results of plankton tows of August 9, 1932, are given in Table VIII. Other samples taken in June, July, and August, which have been analyzed, present a similar picture, hence these data will be used as a basis for the present discussion. As shown in Table VIII, characteristic surface diatoms do not occur in appreciable numbers in the stomach contents of this species. Certain tintinnids such as *Tintinnus subulatus* and *Tintinnopsis campanula* occur in both surface and bottom water layers. Dinoflagellates were found to be relatively scarce at the surface.

TABLE VIII

PLANKTON ORGANISMS FOUND IN DIGESTIVE TRACTS OF *Mya* AND IN WATERS OVER BED FROM WHICH THE CLAMS WERE LATER EXAMINED
(St. Andrews, N.B., August 9, 1932)

Name	Some forms present in		
	Surface tows	Bottom tows	Stomach contents
Chaetoceros (3 species)	very common	common	
Ceratulina	common	rare	
Rhizosolenia semispinosus	common		
Rhizosolenia styliformis	rare		
Pleurosigma		very common	common
Paralia sulcata		common	very common
Navicula		occasional	common
Nitzschia closterium			rare
Dinophysis (with cell contents)			rare
Tintinnus subulatus	common	common	common
Tintinnopsis campanula	common	occasional	occasional
Tintinnopsis cylindrica	rare		occasional
Peridinium crossipes		occasional	very rare

A study of the results obtained from bottom tows over the clam beds has revealed the presence of several species which are also abundant in the surface waters. In addition, *Pleurosigma* sp. and *Paralia sulcata* are quite abundant. These last-mentioned forms constitute the most numerous diatoms present in the stomach contents that were examined. The sample given in Table VIII perhaps lacks the usual variety of occasionals. From another sample collected on August 12 and dominated by *Paralia* and *Pleurosigma*, the following forms are recorded as very rare; *Chaetoceros compressus*, *Coscinodiscus* sp. *Nitzschia*, *Closterium*, *Navicula* sp. (sometimes very abundant) and *Rhizosolenia semispinosa*.

Among the tintinnids of the stomach contents *Tintinnus* (*Helicostomella*) *subulatus* is probably the most abundant species. Frequently the shell contents were absent, which might indicate that they had been digested. However, inasmuch as the clams were either preserved or examined immediately after collection, and also since the diatoms were usually in a well preserved condition, it is believed that many forms of this genus are without cell contents when taken in by the clam.

Dr. Braarud* states that he frequently finds empty tintinnids in plankton collections from this region, which fact supports our contention. Dinoflagellates are represented chiefly by *Dinophysis* sp., often very abundant, and a species of *Peridinium* listed as very rare. As in the case of the tintinnids, specimens of *Dinophysis* sp. are usually without cell contents. The silicoflagellate *Distephanus speculum* has also been observed.

It is seen that diatoms taken in by *Mya arenaria* are bottom forms which are also frequently present in the bottom plankton tows (*Paralia*, *Pleurosigma* and *Navicula*) and neritic species. Although tintinnids, the most abundant zooplanktonts recorded from stomach contents, are commonly present, their numbers are often due to empty shells which were empty when taken in. The data, although scanty in some respects, are indicative of the major role played by diatoms in the food of *Mya arenaria*, during the period studied.

Table IX shows the approximate age of clams belonging to the various length classes at two different levels on the beach at Clam Cove. These age determinations are based on annual-ring measurements of 1932 (Table V). It may be pointed out that whereas a clam at the lower levels is able to reach a marketable size (40 mm. in length) in three or four years, approximately double this period is required at the upper levels.

TABLE IX

APPROXIMATE AGE OF CLAMS BELONGING TO THE SEVERAL LENGTH CLASSES STUDIED IN THE CLAM COVE REGION

Length class, mm.	Approximate age in years at	
	7–9 ft. level	16 ft. level
5 - 14	1 - 2	1 - 2+
15 - 24	2 - 3	3 - 4
25 - 34	3 - 3+	5 - 6
35 - 44	3+ - 4+	7 - 8
45 - 54	4+ - 5+	8 - 10
55 - 64	5+ - 7	probably over 10
65 - 74	7 - 9	

Biotic relations. Several species of Polychaete worms such as *Nereis virens* (about 24 per square metre) and a Nemertine (*Cerebratulus* sp.) in small numbers were recorded. A few small communities of the sea mussel *Mytilus edulis* occur but not in sufficient numbers to limit the *Mya* population (*cf.* 17).

Little plant life is present on this flat. The surface of the mud is in places covered with diatoms, including *Lichmorpha*, *Navicula* and *Nitzschia*. Occasional tufts of *Fucus vesiculosus* occur, and low down near the mean low tide level in the larger tide-pools some *Zostera marina* is present (18).

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*Assistant Expert for the phytoplankton investigations of the International Passamaquoddy Fisheries Commission.

(b) Extent of Growth

The annual extent of growth has been obtained by summing increments for the five periods which are designated "spring" (111 days), "summer" (59 days), "fall" (57 days) and "late fall" (50 days). Several experiments were continued in 1932 to compare the growth rate during 1931 with that of the corresponding periods of the following year. Measurements made during the period December 3 to 8, 1931, indicate that no appreciable growth took place during the "late fall" period, hence it is believed that during the period from the middle of October to March 1, no significant growth occurred.

TABLE X

TEMPERATURE, IN DEGREES CENTIGRADE, TAKEN AT THE BIOLOGICAL STATION WHARF, ST. ANDREWS, DURING 1931 AND 1932

Months	1931		1932	
	Mean daily temperature		Mean daily temperature	
	Water	Air	Water	Air
January	2.96	−6.42	3.01	−1.98
February	1.71	−5.52	0.71	−7.97
March	1.68	0.72	0.72	−2.91
April	3.72	5.34	3.29	3.34
May	6.63	10.26	6.29	9.57
June	9.63	14.85	9.03	14.46
July	12.37	18.22	11.37	16.46
August	13.23	18.18	13.11	17.58
September	12.23	13.43	12.91	16.04
October	10.93	9.52	11.35	9.12
November	8.58	5.65		
December	4.71	−3.37		

TABLE XI

CONSTANTS OF FREQUENCY DISTRIBUTIONS OF LENGTHS OF SMALL AND LARGE CLAMS USED IN EXPERIMENTS OF 1931

Box	Location	Level, ft.	No. of specimens	Mean length during experiments		Standard deviation	C. V.
				At beginning	At end		
A	Clam Cove	18	45	18.4 ± 0.1		1.04 ± 0.11	5.7 ± 0.6
			45		23.9 ± 0.2	1.31 ± 0.13	5.4 ± 0.5
B	Clam Cove	7	45	18.5 ± 0.1		0.96 ± 0.10	5.2 ± 0.5
			44		30.5 ± 0.4	2.45 ± 0.26	8.1 ± 0.8
C	Clam Cove	5	45	18.4 ± 0.1		0.94 ± 0.09	5.1 ± 0.5
			41		28.6 ± 0.4	2.67 ± 0.29	9.3 ± 1.0
A1	Clam Cove	18	45	26.4 ± 0.1		0.84 ± 0.08	3.2 ± 0.3
			44		31.7 ± 0.2	1.60 ± 0.17	5.0 ± 0.5
B1	Clam Cove	7	45	26.6 ± 0.1		0.97 ± 0.10	3.7 ± 0.4
			42		34.7 ± 0.4	2.77 ± 0.30	8.1 ± 0.9
C1	Clam Cove	5	45	26.5 ± 0.1		0.77 ± 0.08	2.9 ± 0.3
			42		35.2 ± 0.4	2.44 ± 0.26	6.9 ± 0.8
D	St. Andrews	5	45	19.3 ± 0.2		1.24 ± 0.13	6.4 ± 0.6
			44		32.0 ± 0.1	0.71 ± 0.07	2.2 ± 0.2
D1	St. Andrews	13	45	18.3 ± 0.1		0.92 ± 0.09	5.0 ± 0.5
			41		26.3 ± 0.3	1.85 ± 0.20	7.0 ± 0.8
E	St. Andrews	7	45	22.2 ± 0.1		0.89 ± 0.09	4.0 ± 0.4
			45		32.0 ± 0.4	2.82 ± 0.29	8.8 ± 1.0
F	Waweig Creek	13	45	22.3 ± 0.1		0.90 ± 0.09	4.0 ± 0.4
			38		24.3 ± 0.2	1.01 ± 0.11	4.2 ± 0.5

(1) Annual growth

Annual growth records have been obtained by summing the increments of growth for each season. Consequently, they are likely to be somewhat less than the true values on account of the probable retardation in rate of growth caused by the clams being disturbed for the purpose of measurement.

The annual growth increments of small (initial length, 18.4 mm.) and large specimens (initial length, 26.5 mm.) are treated separately. For small clams, the maximum absolute increment of growth obtained was 12.8 mm. (66.2%) for specimens which possessed an initial length of 19.3 mm. (Table XII).

Results obtained at the beaches near St. Andrews and at Clam Cove indicate the influence of beach level on the rate of growth of small clams. Fig. 4, C shows the growth increments for specimens of approximately the

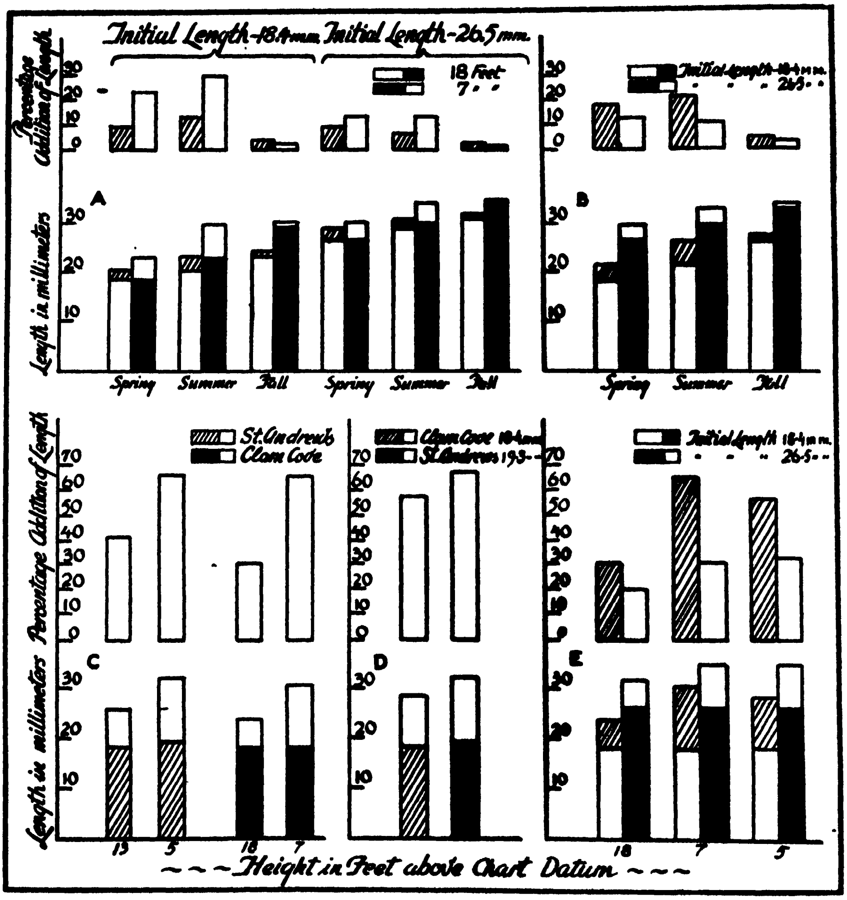

FIG. 4. A. *Seasonal growth of "small" and "large" clams at two levels on beach at Clam Cove, N.B.* B. *Comparison of seasonal growth of small and large clams expressed as absolute increment of length and as percentage addition of length. Figure is based on average records for three levels on the beach at Clam Cove, N.B.* C. *Comparison of annual growth expressed as absolute increment of length and percentage addition of length at different levels on beaches at St. Andrews and Clam Cove, N.B.* D. *Comparison of annual growth of clams of similar initial lengths at the same level (five feet above chart datum) on two beaches located at Clam Cove and St. Andrews, N.B.* E. *Comparison of the extent of growth of small and large clams at three levels of the beach at Clam Cove.*

TABLE XII

INCREMENTS OF SEASONAL GROWTH OF SMALL AND LARGE CLAMS USED IN EXPERIMENTS OF 1931

Box	Location	Level, ft.	Initial mean length, mm.	Amount of spring growth, mm.	Addition of length during spring, %	Amount of summer growth, mm.	Addition of length during summer, %	Amount of fall growth, mm.	Addition of length during fall, %	Total growth, mm.	Addition of length during entire period, %
A	Clam C.	18	18.4	1.8	9.7	2.7	13.4	1.0	4.5	5.5	30.0
B	Clam C.	7	18.5	4.3	23.5	6.9	30.0	0.9	3.0	12.1	65.3
C	Clam C.	5	18.4	3.8	20.9	4.6	20.8	1.9	7.2	10.4	56.5
A1	Clam C.	18	26.4	2.6	9.6	1.9	6.6	0.8	2.7	5.3	20.0
B1	Clam C.	7	26.6	3.7	13.7	4.1	13.5	0.4	1.3	8.2	30.7
C1	Clam C.	5	26.5	4.1	15.4	3.2	10.4	1.6	4.9	8.9	33.5
D	St. And.	5	19.3	5.9	30.5	4.7	18.8	2.1	7.2	12.8	66.2
D1	St. And.	13	18.3	3.0	16.3	3.4	15.6	1.3	5.1	7.7	41.8
E	St. And.	7	22.2	3.5	15.7	3.7	14.5	2.6	8.9	9.8	44.4
F	Waweig C	13	22.3	1.6	7.4	0.4	1.6	0.0	0.0	2.0	9.2

same initial length planted at different levels (5 and 13 ft. above chart datum) on beaches located in the St. Andrews vicinity. The percentage addition of length at the upper level was 35% less than that obtained at the five-foot level.

The annual absolute increment of growth in Box B (Clam Cove) at a level of seven feet above chart datum was 12.1 mm. (65.3%). A comparison of these results with those obtained for specimens of similar initial lengths planted in the same beach but at a different level (18 ft.) reveals a reduction of over 50% in absolute as well as percentage increments (Table XII and Fig. 4, C). The total absolute increment during 1931 and 1932 (until the end of the summer period) at the eighteen-foot level was 9.7 mm. (52.8%) in comparison with 19.0 mm. (102.9%) at the seven-foot level (Table XIII). A confirmation of this reduction in growth rate accompanying decrease in level has been demonstrated by the results obtained from annual-ring measurements of growth in nature (page 104).

Data obtained from experiments in Boxes C and D have indicated that under comparable conditions the rates of growth on two different beaches near the Biological Station are of a similar order of magnitude.

The maximum annual growth of specimens 26.5 mm. long (large clams) was 8.9 mm. (33.5%) and took place at a level of five feet on the beach at Clam Cove—Box C 1 (Table XII).

The influence of exposure on the rate of growth is shown by comparing the growth in Box C 1, 8.9 mm., with that in Box A 1, 5.3 mm., located 18 ft. above chart datum (Table XII). Again, as in the case of the small clams, a reduction in rate of growth accompanying increase in level is evident, but here the decrease is not so conspicuous. The reduction in percentage addition of length is about 30% as compared with 50% for the small clams.

The experiments, which were continued throughout the spring and summer periods of 1932, showed the total absolute increment of growth to be 8.5 mm. (32.3%) at the higher level, whereas at the seven-foot level (Box B) the increments were 14.5 mm. and 55.0% respectively (Table XIII).

Growth increments of small and large clams planted at the same and different beach levels indicate a comparatively slow rate of growth of the large clams (26.5 mm.). The annual growth increments for small and large clams at three levels are given in Table XII and Fig. 4, E. The differences observed in those growing at levels of five and seven feet are not large, the increment of growth in Box D (level, five feet) being 1.6 mm. less than that in Box B. This is due to excess of surface silt at the lower level. (See page 122).

In general, it may be said that the rate of growth of *Mya arenaria* in the Bay of Fundy region is small and the growing period of short duration.

(2) Seasonal growth

A study of the trend of growth throughout each season is of interest from the viewpoint of the fluctuation in environmental conditions, especially temperature and food. The growth rates of small and large clams are presented separately.

TABLE XIII

AMOUNT OF SEASONAL GROWTH DURING 1931 AND 1932 AT CLAM COVE, N.B.

Seasons	Average length at end of each season, mm.	Seasonal absolute increment of growth, mm.	Seasonal addition of length, %	Average length at end of each season, mm.	Seasonal absolute increment of growth, mm.	Seasonal addition of length, %
	Initial length 18.4 mm. (Year 1931)					
	Box A—Level 18 ft.			Box B—Level 7 ft.		
Spring	20.1	1.8	9.7	22.8	4.3	23.5
Summer	22.9	2.7	13.4	29.7	6.9	30.0
Fall	23.9	1.0	4.5	30.5	0.9	2.9
Total		5.5	30.0		12.1	65.3
	(Year 1932)					
Spring	26.9	2.9	11.9	34.1	4.0	13.5
Summer	28.2	1.3	4.9	37.1	2.9	8.6
Total for 1932		4.2	17.4		6.9	23.0
Total for 1931 and 1932		9.7	52.8		19.0	102.9
	Initial length 26.4 mm. (Year 1931)					
	Box A1—Level 18 ft.			Box B1—Level 7 ft.		
Spring	28.9	2.5	9.6	30.2	3.7	13.7
Summer	30.9	1.9	6.6	34.3	4.1	13.5
Fall	31.7	0.8	2.7	34.8	0.4	1.3
Total		5.3	20.0		8.2	30.7
	(Year 1932)					
Spring	33.9	2.2	6.9	37.6	3.2	9.3
Summer	34.9	1.0	3.0	40.7	3.1	8.4
Total for 1932		3.2	10.1		6.4	18.5
Total for 1931 and 1932		8.5	32.3		14.5	55.0

The seasonal distribution of growth of small clams under favorable conditions, is shown by the results obtained in Box B (Tables XII and XIII and Fig. 4, A). The maximum seasonal growth occurs in summer, spring growth occupying second place; the difference, in this instance, being 2.6 mm. (6.5%). The fall growth is very small, being less than 1 mm. (3.0%) as compared with 6.9 mm. during the summer season. This phenomenon has been found to be of quite general occurrence in the Passamaquoddy region.

In Table XII and Fig. 4, A the relative increments of seasonal growth at different levels are shown for both small and large specimens. Considering at first the small clams, the greatest growth occurring at levels of 7 ft. and 18 ft.

is during the spring and summer seasons. In the fall, the largest percentage addition of length, 7.2, was obtained at the five-foot level. To throw more light on the relative seasonal variations in growth rate for this region, Fig. 4, B, based on average records for the three levels, 5, 7 and 18 ft., is presented. The figure shows the average seasonal absolute increments as well as the average percentage additions in length. The extent of growth during the spring and summer seasons is practically identical (addition of length about 20%), whereas, in the fall it is reduced to less than 5%.

Fig. 5, C shows the seasonal growth obtained in small clams of similar initial lengths (18.3–19.3 mm.) on nearby beaches. In Box D1 planted at the 18-foot level, spring and summer increments are quite similar, 3.0 mm. and 3.4 mm. respectively, whereas during the fall period the length increment is only 1.3 mm. At the lower level the growth rate is greater during all seasons, especially spring.

The Clam Cove experiments with both the small and large specimens at levels of 18 and 7 ft. above chart datum were continued during the spring and summer periods of 1932. The total amount of growth during these seasons in the case of small specimens at the 18-foot level was 4.2 mm. (17.4%) and at the lower level, the absolute increment was 6.9 mm. (23.0%). These results indicate a somewhat slower rate than during the same period of 1931 (Table XIII).

Comparing the spring increments obtained during 1931 and 1932, little difference is observed whereas during the summer period the rate was somewhat less during 1932 (Table XIII). In order to compare the summer increments during 1931 and 1932, a box containing 45 clams possessing a mean length of 30.1 mm. was planted at a level of seven feet above chart datum at Clam Cove. The absolute increment obtained for the summer period was 3.0 mm. (9.9%). The values obtained in a comparable experiment during the same period of 1931 were 4.1 mm. and 13.5% respectively (Table XIV). These results suggest that the growing conditions in Clam Cove during the summer of 1932 were slightly less favorable for *Mya* than in the corresponding period of the previous year. Other data presented in another part of this paper corroborate these results. A comparison of the spring and summer increments during 1932 for specimens possessing similar initial lengths, namely, 34.4 and 34.1 mm., shows a slight retardation during the summer season (Table XIV).

A comparison of the seasonal amount of growth of small clams, 18.4 mm., taken from the same level on different beaches is presented in Table XII and Fig. 5, A. Comparing the percentage additions of length it is seen that, with the exception of the spring season, results are almost identical.

An examination of the results of the seasonal growth rate of large clams, 26.5 mm., indicates a growth of 4.1 mm. (Box C 1) in the spring at a level of five feet. This is the maximum absolute increment of length obtained for any single season. The smallest amount of growth for this length class occurred in the fall season when the absolute increment was 1.6 mm. (Table

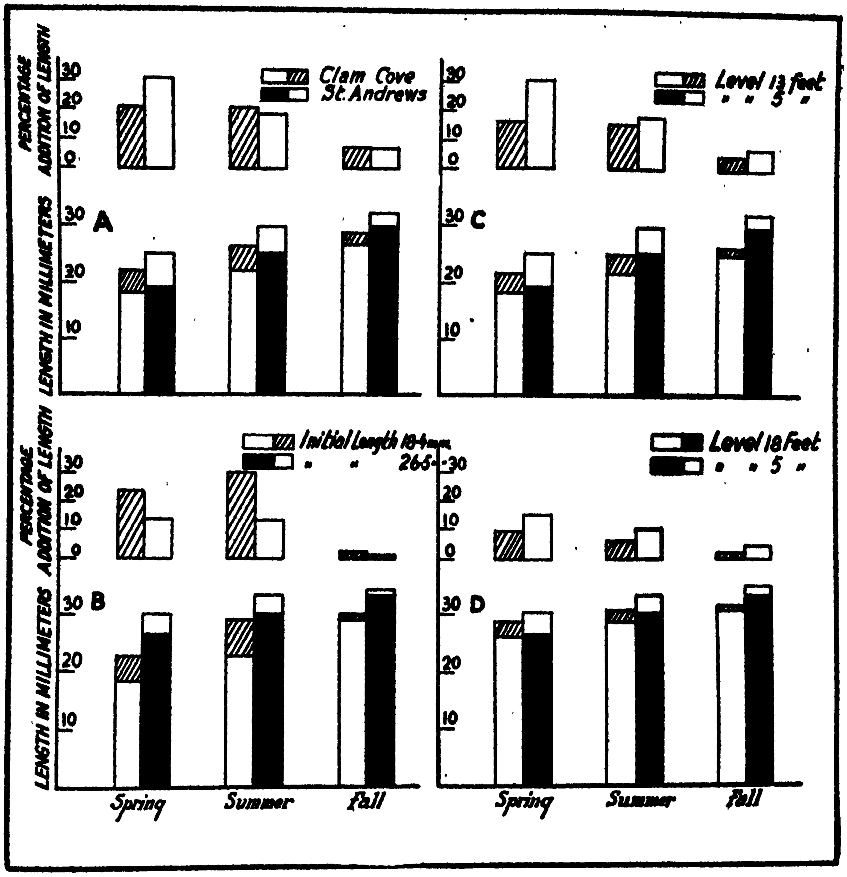

FIG. 5. A. *Comparison of seasonal growth of clams of similar initial lengths at the same level on beaches at St. Andrews and Clam Cove, N.B.* B. *Comparison of seasonal growth of "small" and "large" clams at a level of seven feet above chart datum on beach at Clam Cove, N.B.* C. *to* D. *Comparison of seasonal growth of clams of similar initial lengths at two different levels on beach at St. Andrews* (C) *and Clam Cove* (D).

XII and Fig. 4, A). As is shown in Fig. 4, B, the percentage of growth in spring is slightly in excess of the summer growth; however, both are far greater than the fall increment.

The rates of growth of large clams on different levels of the same beach, are indicated in Table XII and Figs. 4, A, 4, E and 5, D. As noted above, growth is less at the upper levels throughout each season. The greatest differences were obtained during the spring and summer seasons.

A comparison of the seasonal growth of small and large clams at the same level under favorable conditions is presented in Table XIII and Fig. 5, B. The spring and summer increments are approximately the same in the large specimens, whereas the small clams evidenced a somewhat larger percentage addition of length (30.0%) during the summer season (Boxes B and B 1). A further comparison of growth rate throughout the three seasons in the small and large clams is presented in Fig. 4, B. This figure indicates that the percentage additions of length of both sizes are of similar magnitude during spring and summer and that the growth of the small clams exceeds that of the larger specimens throughout each season.

TABLE XIV

COMPARISON OF THE EXTENT OF GROWTH OF *Mya arenaria* DURING THE SPRING AND SUMMER PERIODS OF 1931 AND 1932

Year	Spring			Summer			Total absolute increment of length, mm.	Total percentage addition in length
	Length at beginning of period, mm.	Absolute increment of length, mm.	Percentage addition in length	Length at beginning of period, mm.	Absolute increment of length, mm.	Percentage addition in length		
1931	26.4	2.5	9.6	28.1	1.9	6.6	4.5	16.9
1932	24.1	2.9	11.9	26.9	1.3	4.9	4.2	17.3
1932	34.4	3.2	9.3	34.1	2.9	8.6		
1931				30.2	4.1	13.5		
1932				30.2	3.0	9.9		

The 1931 experiments with large specimens at levels 7 and 18 feet at Clam Cove, continued during the spring and summer periods of 1932, indicate a general retardation in rate accompanying an increase of size. As a consequence, the differential rates for the two levels exhibit less variation and, moreover, this variation is further minimized by the lower initial lengths at the upper level tending to give a more rapid rate of growth which affects, to a marked degree, the retarding influence of exposure. The total absolute increment obtained for both periods of 1932 at the 18-foot level is 3.2 mm. (10.1%) as compared with 6.4 mm. (18.5%) at the 7-foot level. It is apparent that the retarding influence of exposure is not greatly affected by the difference in initial length of 3.7 mm. At the 18-foot level, the increments for the spring period were about double those for the summer season (Table XIII) whereas, at the lower level, the variation was much less pronounced.

For the purpose of comparing the spring growth in nature during 1932, at these levels, with that occurring in the experimental boxes, clams of various sizes were selected for measurement and grouped according to the ranges listed in Table XV. By recording the total length and the length of the 1931-1932 ring for each specimen, it was possible to calculate the mean spring increment for clams falling within the various ranges. The purpose of this experiment was to extend our knowledge of seasonal growth rate over a wider range of size and also to determine the difference, if any, between the amount of growth in nature and that in experimental boxes.

From Table XV (18-foot level) it is seen that the maximum percentage addition of length for the sizes considered occurs in those ranging from 8·0 to 11.9 mm., the mean length being 9.6 mm. For the larger sizes, there is a pronounced decline in percentage increments, the percentage addition for specimens having a mean initial length of 38.0 mm. being 2.6. Comparing these results with those obtained by using planting boxes (Table XVI) it is shown that for an initial length of 29.0 mm. at the 18-foot level, the percentage increment was 6.9 whereas, in the planting boxes, the addition was 6.9% when the initial length was 31.4 mm. Again, comparing results obtained from Box A (initial length, 24.0 mm.) with the percentage increment in nature for specimens having an initial length of 25.5 mm., less agreement is found, the difference being 2.9%.

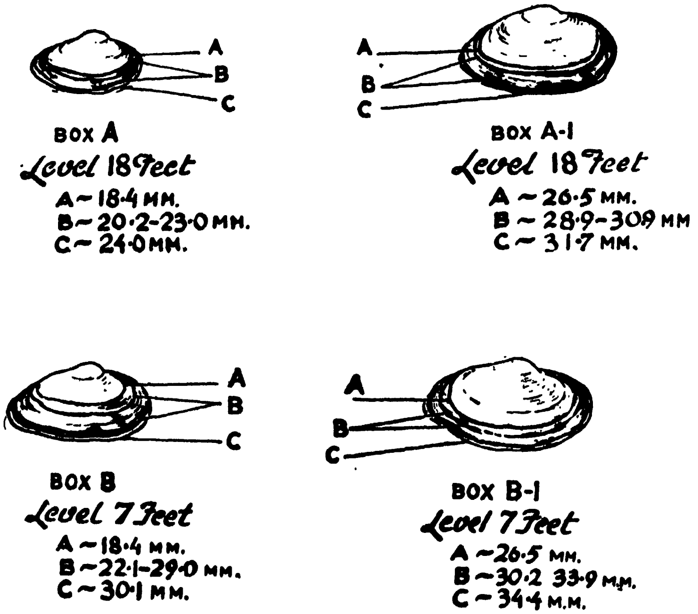

FIG. 6. *Mya arenaria. Extent of growth of small and large sizes at two levels of beach at Clam Cove during experiment in 1931.* A. *Initial length.* B. *Length at end of "spring" and "summer" seasons.* C. *Length at end of experiment. Each figure, reduced about one-third, represents mean type of lots of 45 specimens.*

Considering in a comparative way (Table XV) the data obtained at the lower level (7 feet), a gradual decline in percentage increment of growth accompanying increase in size is observed, the addition being 5.7% for clams possessing an initial mean length of 49.4 mm. In nature, clams possessing an initial length of 29.9 mm. show a percentage increment of 13.1 as compared with 13.3 for specimens having an initial length of 30.1 mm. planted in Box B (Table XVI). For the size used in Box B 1, a fairly good agreement has also been obtained.

Comparing the spring growth rate at levels 7 and 18 feet, a similar relationship is found to that indicated by annual-ring measurements for these levels (Table V), the rate of growth at the eighteen-foot level being reduced approximately 50%. The data presented in Table XV support the results of the planting experiments as well as the conclusion on differential growth rate at the two levels and, furthermore, broaden the picture of growth in nature during the spring season of 1932.

TABLE XV

EXTENT OF SPRING GROWTH DURING 1932 AT CLAM COVE, N.B. AS SHOWN BY ANNUAL-RING MEASUREMENTS

No. of specimens	Range	Length at beginning of spring period, mm.	Amount of spring growth, mm.	Addition in length during spring period, %
			Level; 18 feet above chart datum	
25	8·0 - 11.9	9.6	3.1	32.6
24	12.0 - 15.9	13.8	2.6	18.8
20	16.0 - 19.9	17.4	2.0	11.6
36	20.0 - 23.9	22.3	2.5	11.3
36	24.0 - 27.9	25.5	2.3	9.0
12	28.0 - 31.9	29.0	2.0	6.9
13	32.0 - 35.9	33.8	1.2	3.5
14	36.0 - 39.9	38.0	0.9	2.6
			Level; 7 feet above chart datum	
40	24.0 - 27.9	26.4	4.0	15.2
50	28.0 - 31.9	29.9	3.9	13.1
36	32.0 - 35.9	33.6	2.6	7.9
30	36.0 - 39.9	37.5	2.7	7.3
17	40.0 - 43.9	41.9	2.8	6.7
17	44.0 - 47.9	45.5	2.7	5.9
10	48.0 - 51.9	49.4	2.8	5.7

On a previous page, reference has been made to the decline in growth rate with increase in size, the most marked retardation occurring during the early years (Tables II, IV and V). A similar phenomenon is evident from the results of spring growth presented in Table XV. In the case of clams having initial lengths of 9.6 mm. and 22.3 mm., the reduction of percentage increment was approximately two-thirds (from 32.6 to 11.3%) for the spring season. In Table V it is seen that specimens possessing similar initial lengths also exhibit a reduction in percentage increment of about 60% during a one-year period. Other initial lengths are not such as to permit direct comparison. However, the general trend of diminishing increment is similar in both sets of data.

TABLE XVI

COMPARISON OF THE EXTENT OF GROWTH IN NATURE AS SHOWN BY ANNUAL-RING MEASUREMENTS WITH THAT IN PLANTING EXPERIMENTS AT CLAM COVE, N.B. DURING SPRING PERIOD OF 1932

Growth in	Initial length, mm.	Absolute increment of growth, mm.	Percentage addition of length
Nature	29.0	2.0	6.9
Planting experiment	31.4	2.2	6.9
Nature	25.5	2.3	9.0
Planting experiment	24.1	2.9	11.9
Nature	29.9	3.9	13.1
Planting experiment	30.1	4.0	13.3
Nature	33.6	2.6	7.9
Planting experiment	34.4	3.2	9.3

The character of the decline in growth rate with increase in size for the 7-foot level is likewise comparable (Table V and XV). During the spring season of 1932, the percentage increment was 6.7 for clams having an initial length of 41.9 mm., *i.e.*, about 50% of the corresponding addition for specimens with an initial length of 29.9 mm. In Table V, the reduction for clams possessing similar initial lengths is shown to be 45.7% (from 38.3% to 19.8%) during one year.

These results are of particular value in strengthening our position with regard to the relative decline in growth accompanying increase in length.

In connection with seasonal growth rate, it is of interest to note the growth which occurred on the beach at Harbor de Lutre, Campobello Island, during the period from July 9 to September 24, 1931 (78 days) (Table XVII). Clams were planted at a low level on the flat and the average amount of growth per specimen was 4.2 mm. (15.9%). These results, although not directly comparable with the Clam Cove data on account of the difference in the time factor, nevertheless suggest that the growth rate is quite similar on the two beaches.

TABLE XVII

CONSTANTS OF FREQUENCY DISTRIBUTIONS OF LENGTHS OF *Mya arenaria* USED IN EXPERIMENTS AT CAMPOBELLO ISLAND, N.B. (LEVEL: 7 FEET ABOVE DATUM)

Time	No. of specimens	Mean length, mm.	Standard deviation	C. V.	Mean width, mm.	Standard deviation	C. V.
(Location—Harbor de Lutre)							
Planted July 9	45	26.3 ± 0.1	0.78 ± 0.08	2.9 ± 0.3	16.7 ± 0.1	0.69 ± 0.07	4.1 ± 0.4
Removed Sept. 24	43	30.5 ± 0.3	2.14 ± 0.22	7.0 ± 0.8	19.0 ± 0.2	1.10 ± 0.11	5.8 ± 0.4
(Location—Cranberry Point)							
Planted July 7	45	26.3 ± 0.1	0.78 ± 0.08	2.9 ± 0.3	16.6 ± 0.1	0.69 ± 0.07	4.2 ± 0.4
Removed Sept. 24	37	29.3 ± 0.3	2.07 ± 0.24	7.1 ± 0.8	18.5 ± 0.2	1.42 ± 0.16	7.7 ± 0.8

Some interesting results pertaining to seasonal growth were obtained during the fall of 1930 (15). Small specimens (initial mean length, 13.5 mm.) submerged continuously off the Biological Station float, exhibited a growth of about 5.6 mm. (41.4%) during the period August 8 to October 11, 1930 (43 days). Again, specimens (15 in number and 13.6 mm. in mean initial length) planted at a low level on a beach also near the Biological Station showed a similar growth, namely, 5.4 mm. (39.8%) during approximately the same period of time. The last mentioned group of clams was replanted in October and measured again on February 4, 1931. The average growth during this period was 2.7 mm. (percentage addition of length, 14.28). These results are indicative of two main facts, first, that growth during the so-called fall season was, during 1930, significantly more than that of 1931, and second, that growth continued after the middle of October, probably until about December 1, during 1930 (Fig. 8). These results are significant in the light of the small growth after September 1, 1931, and will be discussed in a later part of this paper.

On the basis of the above presentation it is seen that a considerable variation exists with regard to seasonal growth of *Mya arenaria* in the Passamaquoddy region.

2. Limiting Conditions

The complexity of the factors composing a marine environment is such as to render difficult factorial analysis based on field experiments. The results obtained, however, present an expression of the amount of growth occurring in a variety of environments and help to evaluate the relative importance of certain factors in respect to their effect on rate of growth of this form.

(*a*) Description of areas where experimental boxes were planted.

To determine the influence of certain environmental factors (soil, salinity, sulphide, etc.) on the rate of growth of *Mya arenaria*, planting boxes were placed at various levels on the same and different beaches (Table XVIII).

(*b*) Extent of growth.

Three factors exerting a limiting effect on rate of growth have received attention.

(1) *Soil.* Two types of soil which exert a limiting influence on the growth of this species are considered; soft mud with surface layer of silt, and shifting sand. Two lots of clams ($n=45$, initial lengths 26.3 mm.) were planted at Cranberry Point and Harbor de Lutre, Campobello Island, under similar conditions as far as was possible to determine, except that at Cranberry Point the soil was softer and, over the surface, a thin layer of fine silt was very conspicuous (period of experiment, July 8 to September 24, 1931; 78 days). The amount of growth at Harbor de Lutre was 4.2 mm. (15.8%) as compared with 3.0 mm. (11.4%). The difference is probably due to the deleterious mechanical influence of silt on the rate of growth. Practically no clams are present at this level on the Cranberry Point flat, whereas a considerable number occur at Harbor de Lutre where soil conditions are favorable (Table XVII).

Again, a similar deleterious effect is evident in the case of growth in Box C planted at a low level (five feet) on the beach at Clam Cove (Table XII and Fig. 4, E). From an examination of these data, it is seen that with the exception of fall growth, seasonal as well as total difference in percentage additions of length is as high as 9. This decrease in growth rate is correlated with a permanent surface layer of silt, the importance of which, as a factor limiting growth in *Mya arenaria*, is mentioned elsewhere (p. 135).

Shifting sand is an important factor limiting the growth of *Mya arenaria* in the Bay of Fundy region. The almost total absence of a *Mya* population on a certain sand beach located near Joe's Point, St. Andrews, suggested the following experiment carried on during the period August 29 to October 12, 1931, *i.e.*, 44 days (16). When the clams (initial mean length, 14.1 mm.; *n* = 15) were examined at the end of the period only four were alive. Examination of the specimens showed that two had been dead only a short time. All specimens were measured and the mean length value computed was 15.7 mm. The results show that the rate of growth is very slow in spite of the low level of about 10 feet and indicate that the average increment of growth was only about 1.6 mm. (11.4%). Furthermore, the deleterious effect of shifting sand on the survival of a clam population is demonstrated.

TABLE XVIII

DESCRIPTION OF BEACHES WHERE BOXES WERE PLANTED

Box	Beach	Level in feet	Soil type	Salinity per mille of tidal water
A and A1	Clam Cove	18	Fine sand and a little mud	About 31.0
B and B1	Clam Cove	7	Mud and sand ratio about 3 : 1	About 31.0
C and C1	Clam Cove	5	Mud and sand ratio about 8 : 1; considerable silt on surface	About 31.0
D	Near St. Andrews	5	Mud and sand ratio about 2 : 1	About 31.0
D1	Near St. Andrews	13	Mud and sand ratio about 2 : 1	About 31.0
E	Near St. Andrews	7	Mud and sand ratio about 5 : 1; high sulphide content	About 31.0
F	Waweig Creek	12	Mud and sand	0.8—26.0
G	Cranberry Point	7	Mud and sand ratio about 8 : 1; surface layer of silt	About 31.0
H	Harbor de Lutre	7	Mud and sand ratio about 3 : 1	About 31.0

(2) *Salinity.* An experiment to determine the limiting effect of salinity on growth rate in nature was conducted on the intertidal zone of the Waweig Creek at a level of 13 feet above chart datum (Box F) (Table XII and Fig. 7, A). Since it is located several miles from the tidal waters on Passamaquoddy Bay, the range of salinity is very great—from about 30 o/oo (high tide) to about 2 o/oo (low tide), when exposed to ebbing water practically fresh. The chief growth occurred during the spring season, the absolute increment being 1.6 mm. (7.4%) as compared with a total for all seasons of 2.0 mm. (9.2%). The latter figure is less than one quarter of that which occurs under normal conditions at Joe's Point (Box D 1). The seasonal distribution of growth in comparison with that of Joe's Point is shown in Fig. 7, A.

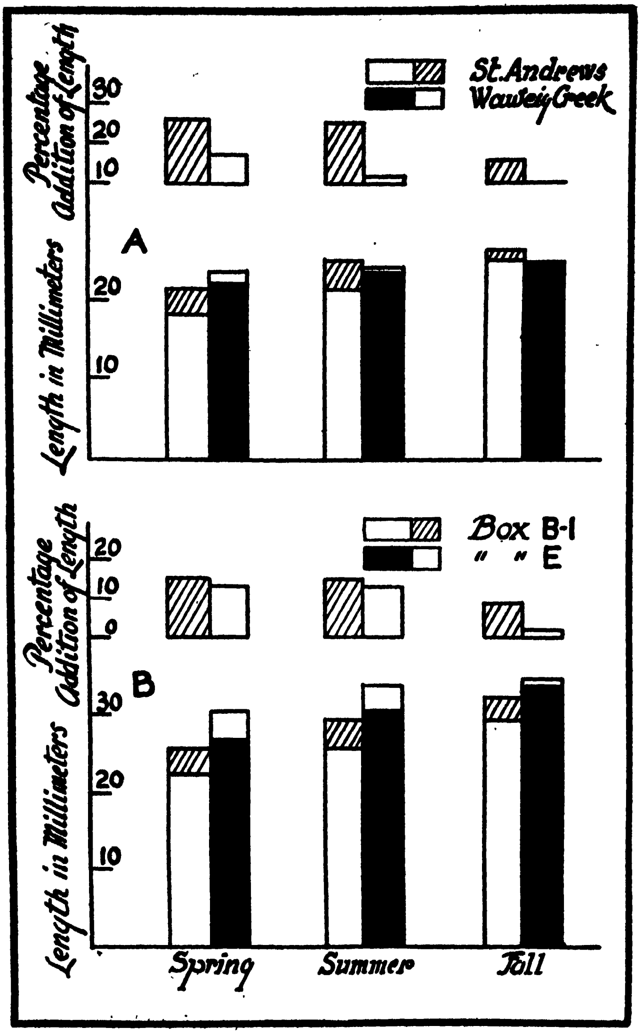

FIG. 7. *Comparison of the amount of growth of Mya arenaria under:— A. a condition of low salinity (Waweig C.) with that under a condition of normal salinity (St. Andrews); B. a condition of high sulphide content (Box E) with that under normal conditions (Box B1).*

A better controlled experiment on the salinity factor was carried out by the writer in the fall of 1930 (September 1 to October 15) at the St. Andrews Laboratory (16). Two lots of small clams ($n = 15$; initial mean lengths, 15.6 mm. and 13.6 mm. respectively) were planted side by side at Brandy Cove, St. Andrews, under similar growing conditions (level and soil) except that one was placed in a small seepage stream and thereby exposed to fresh water. Below the rather shallow layers of beach soil there is a solid ledge of sandstone conglomerate which precludes the possibility of the experiments being affected by fresh subsurface waters. At the end of the experiment, which lasted for 43 days, Box J in the seepage stream contained eleven

TABLE XIX

CONSTANTS OF FREQUENCY DISTRIBUTIONS OF LENGTHS OF *Mya arenaria* USED IN EXPERIMENTS OF 1930. PERIOD; SEPT. 1,—OCT. 15. $N = 15$.

Box I			Box J		
Initial mean length	Standard deviation	C.V.	Initial mean length	Standard deviation	C.V.
15.6 ± 0.2	0.65 ± 0.11	4.2 ± 0.8	13.6 ± 0.1	0.45 ± 0.08	3.3 ± 0.6
Final mean length					
17.4 ± 0.2	0.80 ± 0.15	4.6 ± 0.9	No growth		

specimens which were still alive but which showed no evidence of growth (Table XIX). In the control, Box I, only two clams were dead and the living ones exhibited an average growth in length of 1.8 mm. (11.5%), which is normal growth for this high level. (Compare (22) and (23)).

(3) *Sulphide content.* On the beaches of the Bay of Fundy region it is not unusual to find a black sub-surface layer rich in sulphides and decomposing materials. The influence of this particular condition on the clam fauna is problematical. To ascertain its effect on the growth of *Mya arenaria,* a group of clams (Box E) was planted at a place on the shore near the opening of the St. Andrews sewer to Passamaquoddy Bay (level, 7 feet). The lower layers of soil here were characterized by over 50 volumes per cent of sulphide, a very foul odor, and decaying matter. In spite of these conditions a few clams were found living at a distance of about 0.5 metres from the box.

The amount of growth obtained for the entire period was nearly 10 mm. (44.3%) (Table XII). The result compares quite favorably with the increment obtained elsewhere under favorable conditions (10).

The seasonal growth rate under these conditions is compared with that at a similar level (Box B 1) at Clam Cove (Fig. 7, B). Their percentage additions during the spring and summer seasons are quite similar (Table XII). In the fall, however, a difference in the absolute increment of length of about 2 mm. (7.6%) occurs. This represents the largest fall growth obtained in our experiments during the

TABLE XX

AMOUNT OF GROWTH OBTAINED IN THE SULPHIDE EXPERIMENT

(Box E—level, 7 feet)

Season	Average length at end of each season, mm.	Seasonal absolute increment of growth, mm.	Seasonal percentage addition of length
Year 1931			
Spring	25.7	3.5	15.7
Summer	29.4	3.7	14.5
Fall	32.0	2.6	8.8
Year 1932			
Spring	35.2	3.2	9.9
Summer	37.9	2.7	7.7
Total		15.7	70.7

same period (nearly 9%). The experiment (Box E) was continued throughout the spring and summer periods of 1932. As during 1931, the spring growth slightly exceeded that taking place during the summer period, the absolute increments being 3.2 mm. and 2.7 mm. respectively (Table XX).

From these results, it is apparent that *Mya arenaria* is resistant to a wide range of environmental conditions. In their interpretation, it must be recognized that the so-called limiting factors are not to be considered as isolated environmental units. On the contrary, they are regarded only as the most significant variables and as such must materially modify the incidence of one or more other coexisting factors.

TABLE XXI

SEASONS, THEIR DURATION AND TEMPERATURE,* DURING EXPERIMENTS OF 1931 AND 1932 IN ST. ANDREWS REGION

Season	Duration of season	Number of days	Mean daily temperature, 1931		Mean daily temperature, 1932	
			Water, °C.	Air, °C.	Water, °C.	Air, °C.
Spring						
Total period	March 1 to June 19	111	4 97	6.95	3.57	4.04
Growth period	May 1 to June 19	50	7.62	8 04	7.19	10.59
Summer	June 20 to August 17	59	12 11	17.97	11.45	16.81
Fall	August 18 to October 14	57	12 31	13.64	12.83	13.99
Late fall	October 15 to December 3	50	9 06	6 30		

**Seasonal mean water temperatures represent the mean of daily mean temperatures based on the average of two readings taken about 8 a.m. and 5 p.m. Mean air temperatures represent the mean of daily mean temperatures obtained by calculating the mean of the maximum and minimum daily temperatures.*

V. Discussion

The results are discussed under two main headings, namely, growth in nature, and experimental field growth.

1. GROWTH IN NATURE

In view of the absence of a satisfactory method for the determination of the age of *Mya arenaria*, an attempt has been made to evaluate the age and rate of growth in nature through a study of periodic (annual) rings on the valves. Difficulty has been experienced in recognizing the first (sometimes the second also) rings on large specimens due to a wearing away of the shell surface in the umbo region. The value for the first ring, namely, 3.9 mm., at Clam Cove (4.8 mm. at the Sissiboo River, N.S.) was obtained by measuring the single ring on specimens spawned in 1931 (Table V). Obviously, this value is not directly comparable with figures presented for succeeding rings since it represents the amount of growth during a single season. The growth during the first season may be expected to exhibit marked variation in the different parts of the Bay on account of the susceptibility of the organism at this early period of development to local variations of the physical environment which at a later stage would be less liable to prove effective. The remaining values are based on measurements of specimens of different ages, hence the increments obtained represent the mean amount of growth under average climatic conditions for the region under consideration. It is not usually possible to apply the "annual ring" method for estimating growth increments after the eighth year, because of the relatively small additions and, in many instances, the indistinct nature of the rings.

"Annual ring" measurements have been made of five collections from four widely separated beaches on the Bay of Fundy. Results indicate fairly similar values for the rings of the different collections with the exception of ring Number 2 of the Clam Cove sample. The value obtained was 15.6 mm. as compared with approximately 20.0 mm. for the other regions (Tables II, V and VI). On the basis of our present knowledge we are unable to explain this difference. (*Cf.* (7) and (11)).

Recognition of the existence of annual rings in *Mya arenaria* has permitted an estimation of the seasonal growth during the spring period of 1932 in the case of clams ranging from 8 to about 50 millimetres in length (Table XV). Although the number of specimens in some instances is small, they are quite reliable on account of the small variation within each group. As previously pointed out in detail, these results corroborate those obtained from planting experiments and clearly demonstrate the reduction in growth rate of approximately 50% with increase in level from 7 ft. to 18 ft. above chart datum. The trend of the decline in percentage increment with increase in size is clearly shown.

The results of annual-ring measurements of collections at levels 7 ft. and 18 ft. at Clam Cove illustrate the following points. Firstly, the growth rate at a low level on the beach is approximately double that occurring at the upper levels, thus corroborating the results of seasonal (spring, 1932) growth under both natural and experimental conditions (Tables XV and XVI). Second, the correspondence between the annual growth during 1931 as shown by annual-ring measurements and that determined by planting experiments is significant and, furthermore, strengthens the validity of seasonal increments obtained experimentally in this investigation. Thus, although the initial lengths are not sufficiently similar to permit a direct comparison of the annual increments obtained in 1931 (Table XII) with the results of annual-ring measurements (Table V), nevertheless a fairly good correspondence is evident.

Growth of small clams (initial length, 18.3 mm.) at the higher level during 1931 was 5.5 mm.; equivalent to a percentage addition of 30.0. Referring to Table V, it is seen that for clams possessing a similar initial length, the growth in nature falls about midway between 42.3% and 25.1%, probably quite close to 30%. For the seven-foot level, the absolute increment of growth in Box B was 12.0 mm., whereas the corresponding increment of growth in nature falls around 11.5 mm. (Table V). From Table XII, it is seen that the percentage increment for large clams (initial length, 26.4 mm.) at the upper level during 1931 was 20. In nature (Table V) for a similar initial length the percentage addition lies between 25.1 and 15.1. Specimens of similar initial length, grown at a low level, show a somewhat less percentage increment than that indicated by annual-ring measurements. However, this difference may be expected since growth results obtained in experimental boxes are likely to be somewhat below normal growth in nature owing to disturbance for purposes of measurement. Third, the amount of spring growth during 1932 as indicated by annual-ring measurements is essentially in agreement with the results obtained from planting experiments (Table XVI).

The rate of growth in this species shows marked variation depending on age. The growth rate during early life decreases very rapidly with a subsequent less rapid decline (*cf.* Fig. 3). A similar trend of growth has been obtained by various authors working with molluscs and several species of fish (*cf.* 29, p. 61).

2. Experimental Field Growth

The complexity of conditions operating in nature which affects the growth rate of this species renders a definite explanation of observed phenomena difficult. However, on the basis of our present knowledge, we may at least offer a tentative interpretation of the differential growth rates evidenced by the results. An interpretation of findings based on experiments in nature, requires a recognition of the complexity of physical as well as biotic factors affecting the rate of growth and survival of the species. To isolate the several individual factors in respect to their influence on rate of growth by simulating natural conditions in the laboratory likewise presents difficulties. Hence, in consideration of these facts, care has been exercised to employ control experiments and to check results whenever possible.

It has been noted that specimens of various sizes exhibit an increase in growth with approach to the low water level (Fig. 3, C). Our results corroborate those first reported by Mead and Barnes (14) in demonstrating the fact that clams of this species submerged in water all the time grow more rapidly than those planted at any level in the intertidal zone. Stephen (28), working on *Tellina tenuis* discovered an opposite relationship between rate of growth and level, correlated with a different method of feeding. He obtained no growth below the low water mark. The variation between these two forms has been attributed to difference in food assimilated. Our results strongly suggest that organic detritus does not constitute a very important part of the food of *Mya arenaria* in this region; rather that the microscopic plant life (chiefly diatoms) present in the water is the dominant source of nourishment (*cf.* 1, 9, 24). Lotsy (13) found that a large percentage of the diatoms present in the stomach contents of *Mya arenaria* is completely digested but that the decaying organic matter shows no indication of being digested. Again, it has been demonstrated that the clam in this region is quite sensitive to variation in exposure and thus, we believe, to changing abundance of food.

Hunt (8) states "such a seasonal fluctuation of their diet may be an immediate factor concerned in the seasonal variation in growth of marine animals . . . . it seems likely that a rapid growth rate is correlated with a diet rich in living organisms." Even if detritus were present in considerable quantities in the stomach contents of *Mya arenaria* (which is contrary to our findings) we have no basis for assuming that it is digested, since Yonge (33) failed to find any evidence of the presence of cytase in this species.

The results obtained from a study of annual rings and from field experiments indicate the effect of size on relative growth rate in *Mya arenaria.*

It has been observed that variations occur in seasonal growth on different beaches as well as various levels of the same beach. Furthermore, results point to a variation in duration and amount of growth during the fall and winter of 1930 as compared with the corresponding seasons of 1931. It may be in order to consider first the more general aspects of the findings pertaining to distribution of growth throughout the year 1931, followed by a more detailed discussion of the variation between the several levels on the beach as well as a comparison of the growth increments during corresponding seasons of 1931 and 1932.

In 1931 the seasons of greatest growth were spring and summer. With hardly an exception (note Box E) the length increment after about August 20 (fall season) is relatively small. This fact is clearly shown in Fig. 4, A, in which the seasonal growth of small and large clams is compared. During the late fall period, no significant growth occurred. These data are seemingly contrary to expectations in view of the growth occurring during the spring period when the mean water temperature was comparatively low, namely, 7.6° C. (Table XXI). The spring period, extending from about March 1 to June 19, although much longer than any one of the other periods, probably represents similar time for growth, 50 days. However, on the basis of previous work it appears unlikely that much growth takes place before March 1. Thus, the mean water temperature during April, 3.7° C., was relatively low for growth; in May it was 6.6° C. and in June, 9 6° C. (Table X). Belding (1) states that *Mya arenaria* continues to grow until a temperature of 42° to 40° F. (5.6° to 4.4° C.) is reached. Hence, in view of the low mean temperature for April (3.7° C.), we believe that growth did not start until May and, therefore, results for the spring season represent growth occurring after May 1 (50 days) during which time the mean daily water and air temperatures were 7.6° C. and 8 0° C. respectively (Table XXI). Comparing these mean temperatures with those for the late fall period (also 50 days) it is seen that the water temperature in spring is less while the amount of growth is in every instance far greater (no appreciable growth was obtained for the late fall season). The air temperature is 1.7° C. less during the late fall period. This fact is of little consequence here, especially in the case of clams planted at the lower levels with a very short period of exposure.

Again, comparing results for summer and fall periods of similar length, the extent of growth during summer far exceeds that taking place during the fall period although the water temperatures are similar (12.1° and 12.3° C.). The air temperatures exhibit some variation but not sufficient to serve as factors explaining the differential growth rate.

Considering the magnitude of growth and thermal conditions during the spring and summer periods, there is a general similarity in growth rate but a wide variation in mean daily temperature of both water and air (Table XXI). These facts definitely rule out temperature as the direct determining factor.

The data indicate that some factor besides temperature must be operating to cause these seasonal variations in rate of growth. The question arises as to why the growth rate diminished in September and October and ceased almost entirely during the late fall period. This question can not be definitely answered, however certain lines of evidence are available which support the contention that food supply is largely responsible for the differential rate.

First, mention may be made of the investigations of Kellogg (9) and Belding (1), both of whom consider rate of growth in *Mya arenaria* to be directly correlated with the quantity of food. Wilton and Wilton (32) present evidence to suggest that clams are quite sensitive to variability in their food supply. Second, the seasonal fluctuations in diatom abundance evidence a significant correspondence with growth variability. The intention here is to point out some of the more cogent facts relative to diatom abundance that throw light on the problem in question.

It is stated in a previous paragraph that during the fall period of 1931 the amount of growth was significantly less than that of the corresponding period for 1930. Furthermore, growth continued until about December 1 during 1930, whereas practically none occurred after the middle of October, 1931 (late fall period). For an explanation, attention is turned to temperature and food as the most likely sources.

Regarding temperature, it has been found that the mean daily water temperature during September, 1930, was 13.4° C. as compared with 12.2° C. during September, 1931 (Table X, Fig. 8). As is shown in Fig. 8, the temperatures during the remaining fall months are similar for both years. It seems apparent, therefore, that temperature is not the explanation for the small amount of fall growth during 1931. Likewise, Lea (11) working on seasonal growth rates in herrings clearly points out that these rates are not correlative with the temperature. He states "comparing two periods with approximately the same temperature, *e.g.*, June and October, it is seen . . . . when the temperature in October becomes the same as in June when the rate of growth reached its maximum, the rate of growth is well on its way to the minimum".

In considering the food factor, it may be pointed out in the beginning that sufficient data are not available pertaining to the seasonal or annual abundance of diatoms in the immediate vicinity of the clam beaches under consideration, to warrant definite conclusions. We are particularly fortunate in having access to qualitative and quantitative records collected over a period of several years, by Doctor Viola M. Davidson.

The data which have been obtained from Dr. Davidson were collected at Station 6, located near the middle of the St. Croix River about 800 metres from the clam beaches near the Biological Station, St. Andrews. From the viewpoint of their effect on the abundance of diatoms, conditions operating at this station may be considered similar to those in the waters over the clam beds, owing to the uniformity of nutrient salts at the various depths produced by the intensive mixing characteristic of this region. Thus, with an absence of pronounced stratification such as is encountered in the Gulf of Maine waters, we can reasonably expect the demersal and neritic forms to be affected in about the same way as the deeper water oceanic species*. From these data there has been prepared a logarithmic curve of diatom abundance during the periods of our experiments of 1930 and 1931 (Fig. 8). According to Dr. Davidson, the curve of diatom abundance for 1930 is exceptional, the fall figures exceeding those of any year since 1924. The 1931 curve depicts a somewhat different trend throughout the seasons. It is seen that there was a pronounced decrease during the fall and late fall periods of 1931 which is directly correlated with a pronounced decrease in growth rate. The small amount of growth that is apparent from our results for the period from the

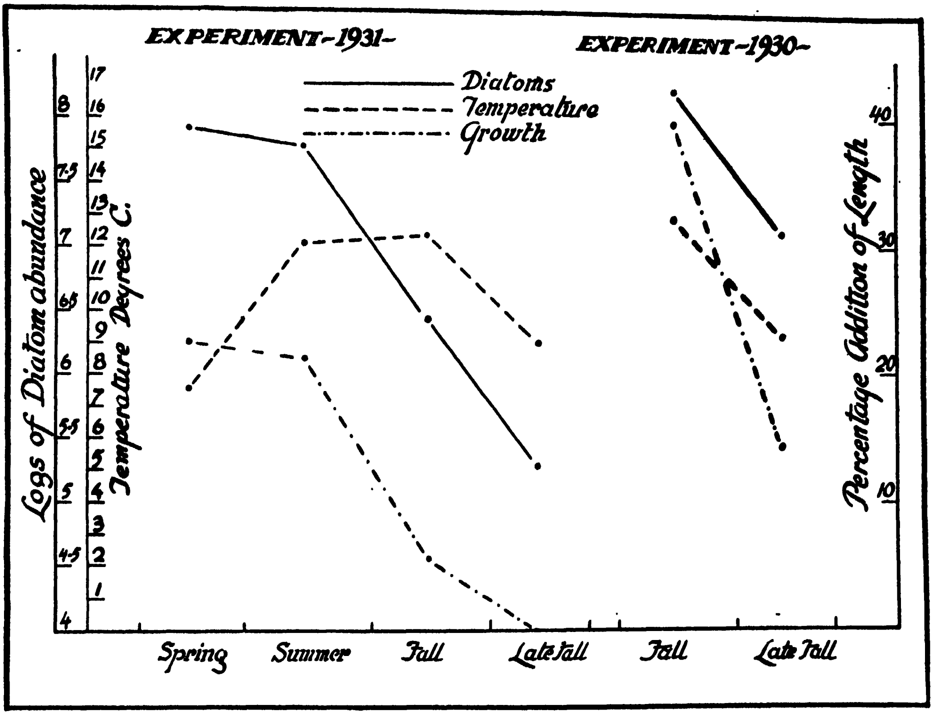

FIG. 8. *Fluctuations in diatom abundance, temperature, and rate of growth of Mya arenaria during periods of experiments of 1930 and 1931. The diatom curve is logarithmic, being based on the geometric means of weekly records obtained at Station 6, St. Croix River, N.B.*

**Personal communication from Dr. Braarud.*

middle of October, 1930, to about March 1, 1931 (2.8 mm.) is apparently due to the low temperatures following December 1, since diatoms were abundant during February (4).

We have considered evidence pertaining to the importance of food in accounting for the difference in rate of growth during the fall periods of 1930 and 1931. This has suggested its possible importance in affecting the seasonal growth rate during 1931.

Comparing the growth curve with the curve of diatom abundance for 1931 (Fig. 8), similar trends are observed. It is noted that the peak of diatom abundance corresponds with the rapid growth of the spring season (the principal growth during this season is considered to occur in May. See above). The average diatom abundance during the summer season, in which growth is quite similar to that of the spring period, is fairly high, and it is very low in the late fall period when growth is approximately nil. On the basis of the diatom abundance, a somewhat higher rate of growth during the fall period might be expected. Growth data for the spring and summer periods of 1932 also show a rapid rate which likewise corresponds with a similar trend of diatom abundance.

An attempt to confirm the importance of diatoms as food was undertaken during the summer of 1932. As a result of qualitative comparisons (quantitative data only relative) between the planktonts of the surface and bottom layers of water and those of the stomach contents of this species, it has been demonstrated that bottom forms which are also frequently found in the plankton (*Paralia sulcata*, *Pleurosigma* sp. and *Navicula* sp.) and neritic species are most abundant in the stomach contents.

Of the zooplanktonts present, the tintinnids occur in greatest abundance, the common species being *Tintinnus subulatus* and species of *Tintinnopsis*.

To recapitulate, our data indicate that growth, during the months from December to April, inclusive, is practically negligible owing to the mean water temperature (air temperature also) being considerably below 5.0° C. and that seasonal growth during the remaining months of the year is not correlated directly with these conditions, but rather with the food supply.

The average results obtained for the beach at Clam Cove during 1931 (Table XII and Fig. 4, B) show that the percentage additions of length during spring and summer were approximately the same in the case of small and large specimens and that there was, in both sizes, a very evident decrease during the fall of 1931. A corroboration of these indications was obtained in field experiments during the spring period of 1932 (Table XIV). The percentage increment of growth, 9.3 mm., was only slightly higher than that for the summer season (8.6%) when the mean daily temperatures were appreciably higher (Table XXI). According to Dr. Davidson* the abundance of diatoms in the spring period was high and followed a trend quite similar to that of 1931 (Fig. 8) and hence corresponded with the amount of growth obtained. Similar results have been obtained in comparable experiments during the spring seasons of 1931 and 1932. During the summer period of 1932, a somewhat lower rate has been obtained than during the corresponding period of 1931 (Table XIV). The amounts of spring growth during 1932 as evidenced by the results of planting experiments and annual-ring measurements have been directly compared in Table XVI and a very good correspondence obtained. By means of ring measurements it has been possible to present in Table XV a picture of the spring growth in 1932 over a fairly wide range of size in the case of clams growing at both high and low levels of the beach at Clam Cove, N.B.

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*Private communication.

The effect of exposure in limiting seasonal growth on the beach near St. Andrews is shown in Fig. 5, C and D. Annual-ring measurements corroborate the results of planting experiments in pointing to an approximate 50% reduction in annual growth rate accompanying an increase in level from 7 to 18 ft. above chart datum (Tables V and XV). It should be remembered that exposure not only restricts feeding time but also aeration, and therefore metabolic rate. However, this might not prove significant in limiting the growth processes, providing an adequate food supply was available, since Collip (2) was able to demonstrate that clams possess a source of oxygen, which he considers may be a compound in the tissue, of the nature of an organic peroxide, that is available for metabolic needs during long periods of oxygen insufficiency in the enveloping medium. In the case of *Tellina tenuis* which feeds on detritus, Stephen (26) has obtained greatest growths at levels exposed for long periods. Thus, a decrease in aeration does not seem to be detrimental. Our results do not show that detritus is an important source of food for *Mya arenaria* (*cf.* 33). On the basis of these observations, it seems safe to conclude that the restricting effect of exposure is caused not by decrease in aeration, but rather by an accompanying reduction in the available food supply.

(b) Unfavorable Conditions

Important unfavorable conditions in nature which have received attention are soil, salinity, and sulphides.

Soil. Two types of soil which have been found to exert a limiting influence on the growth of *Mya arenaria* are (i) soil consisting of a large percentage of mud mixed with sand and covered with a layer of fine silt and (ii) fine shifting sand.

In the case of the first type, the retardation of growth is attributed to the mechanical action of the fine silt which interferes with the normal feeding processes (*cf.* 33). Results obtained in Boxes G and H planted at Cranberry Point and Harbor de Lutre (difference in percentage addition of length, 4.5) demonstrate the retarding influence. Furthermore, at Clam Cove, specimens in Box C, although planted at a slightly lower level than those of Box B, nevertheless grew at a slower rate throughout the principal growing seasons, the difference in total percentage addition of length for the entire period being 8.8. The most pronounced difference in the absolute increment of growth (2.3 mm.) equivalent to a percentage addition of length of 9.2, occurred during the summer season. Apart from the limiting effect on growth, there has been noted a scarcity of clams in this type of beach (soft mud), indicating an unfavorable condition for survival of the set.

On the beaches consisting of fine shifting sand, clams are not only absent but fail to survive when planted. In the experiment carried on at Joe's Point during a period of 65 days only 27% of the clams survived. This is due to the siphons becoming filled with sand which the clam is unable to remove.

Salinity. That *Mya arenaria* is capable of resisting a wide range of salinity is very well known (1). However, results show that clams growing in nature may evidence a distinct retardation when the range is extreme (2 o/oo to 30 o/oo) (*cf.* 22 and 23).

Sulphide content. Results obtained in the case of clams planted near the mouth of a sewer where the sulphide content of the black soil was over 50 volumes per cent, show, contrary to expectations, a growth quite comparable to that occurring under favorable conditions in Box B 1 at Clam Cove (Table XII and Fig. 7, B). The data obtained indicate the high resistance of *Mya arenaria* to high sulphide concentration which is found on quite a number of beaches. In view of the complexity of the chemical changes operating in the black layers, it is difficult to explain the action of sulphides on the growth processes (*cf.* 18).

VI. Summary and Conclusions

The results of this study may be summarized as follows:

1. GROWTH IN NATURE

1. *Mya arenaria* possesses "annual rings" on the valves which serve as a criterion for the determination of age and rate of growth. Frequently difficulty is experienced in recognizing the first ring which suggests the amount of growth during the first year, owing largely to a wearing away of the outer shell layer in the umbo region.

2. Study of the annual rings shows that growth activity per unit of length exhibits a marked decline with increase in age from a very early period. The percentage additions of length obtained by averaging results from four Bay of Fundy samples are:—during the second year, 346.3; the third year, 59.9; the fourth year, 33.3; the fifth year, 19.6; the sixth year, 14.3; and the seventh year, 10.7.

2. EXPERIMENTAL FIELD GROWTH

3. In the experiments of 1931, clams of two sizes were selected; "small" (17 to 20 mm. in length) about two years old, and "large" (25 to 28 mm. long) three years old. For an understanding of the role played by environmental factors which influence growth, two series of experiments have been carried out under: (*a*) Normal conditions; (*b*) Limiting conditions.

(a) Normal Conditions

4. Under favorable conditions, the factors considered are submergence, food and temperature. The beaches studied are located at Clam Cove, Deer Island, and near St. Andrews. Boxes were planted at three levels: high, 18 to 19 ft. above chart datum; medium, 13 ft.; and low, 5 to 7 ft., on experimental beaches. In general, small clams (18.4 mm. in length) about two years old exhibit a faster rate of growth than large individuals (26.4 mm. long) three years old, throughout each season; the maximal annual absolute increment of growth for small clams was 12.8 mm. (percentage addition of length, 66.2) and 8.9 mm. (percentage addition of length, 33.5) in the case of large clams.

5. A comparison of the growth rate during the third and fourth years, obtained in growth experiments during 1931, with that calculated from the estimated annual rings of specimens growing at comparable levels, has yielded similar results (page 117), namely, an absolute increment of length during the third year of about 13.0 mm. and during the fourth year of approximately 11.0 mm.

6. The rate of growth of small and large clams varies inversely with the beach level. Regarding small clams planted at Clam Cove, the annual growth at a level of eighteen feet above chart datum was less than one-half that occurring at a level of seven feet. In the case of large clams at corresponding levels, the reduction was about one-third.

7. The principal growth took place from the beginning of May until September (spring and summer seasons) when approximately 92% of the yearly growth for small and large specimens occurred. It may be mentioned that the amount of growth during the spring season was similar to that taking place during the summer period. This occurred in the case of both small and large clams, *e.g.*, the percentage additions of length during each of the aforementioned seasons for small clams in Box C was about 20, for large clams in Box B 1 about 13.

8. The amount of growth during the spring seasons of 1931 and 1932 was quite similar, whereas in the case of the summer periods, a slight decline was shown during 1932.

9. Very little growth occurred during the fall of 1931 in comparison with the corresponding season of 1930 when growth continued until about December 1 (Fig. 8).

10. The following explanations are offered for the variations in seasonal growth which occurred during 1930 and 1931, as well as the difference in fall growth during both years:

(*a*) Practical absence of growth during the winter season (about December to April, 1931) is attributed chiefly to low temperature.

(*b*) Variation in growth throughout the entire year at different levels is directly correlated with the time of submergence. Thus, in 1930, clams submerged all the time showed greater growth than those planted at any level on the intertidal zone.

(*c*) Variation in the amount of growth during the different seasons of 1931 and that in the fall seasons of 1930 and 1931 is attributed chiefly to fluctuations in the amount of food (diatoms) present. According to the investigation of Doctor V. M. Davidson, the quantity of diatoms in plankton of the Passamaquoddy region in the fall of 1930 was exceptionally high as compared with that of 1931 (Fig. 8). Also, the abundance of diatoms during the spring and summer months of 1931 was much greater than during the fall of this year, thus indicating a close correspondence with the known variations in rate of growth. Available data for the spring and summer periods of 1932 indicate a similar trend.

11. Of the plankton constituents in the stomach contents of *Mya arenaria*, diatoms have been found to be most abundant, and zooplanktonts, chiefly tintinnids, play a minor food role. The diatoms taken in by *Mya* are, in the main, bottom forms which also occur frequently in the plankton (*Paralia sulcata*, *Pleurosigma* sp. and *Navicula*) and neritic species.

12. Experiments designed to analyze the resistance of *Mya arenaria* to unfavorable conditions of soil, salinity, and sulphide content were set up on beaches especially suitable for this purpose (Table XVIII).

13. Two types of soil limit the growth rate of this species (a) fine shifting sand, (b) soil consisting of a high percentage of mud with a surface layer of fine silt.

14. Water possessing a very low salt content is unfavorable for growth. However, the resistance of *Mya* to this factor is so great that the usual range of salinity encountered on commercial beaches of the Bay of Fundy region cannot be considered an important factor in determining differential growth rate.

15. The growth rate of clams is not significantly retarded by the sulphide content present in the soil of clam beaches of the Bay of Fundy.

Acknowledgments

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STUDIES ON THE ENDOPARASITIC FAUNA OF TRINIDAD MAMMALS

II. ADDITIONAL PARASITES FROM TRINIDAD DEER

BY THOMAS W. M. CAMERON

Abstract

Setaria bidentata (Molin) and *Eucyathostomum longesubulatum* Molin, are recorded from *Mazama simplicicornis* and are re-described together with a species of Oxyurid from the same host.

In a previous article in this Journal (1) a number of helminth parasites from a deer from Trinidad were described. Thanks to the co-operation of Professor Urich, the entrails of a second deer of the same species (*Mazama simplicicornis*) have recently been received from Trinidad and a careful examination has revealed the presence of three species of nematodes. Two of these appear to be old forms described briefly by Molin in 1858 and 1861 from deer in Brazil and not since seen, while the third appears to be unrecorded.

Setaria bidentata (MOLIN, 1858) RAILLIET ET HENRY, 1911

Two males and three females were recovered from the omentum in a good state of preservation. The cuticle is longitudinally striated with many fine lines which, however, are absent from the lateral areas of the body. The head is rounded and not separate from the remainder of the body. The peribuccal ring is prominent (Figs. 1–4) but is notched only laterally to give the appearance of single dorso-ventral teeth. Each tooth is complete and is *not* indented. The peribuccal ring is surrounded by the normal six circum-oral papillae. The appearance is similar in both sexes, although the ring is more conspicuous in the female than in the male.

The females have an average length of 75 mm. and a maximum thickness of 0.8 mm. The oesophagus is divided into the usual two parts; the anterior, more muscular portion being about 0.56 mm. long, the posterior more glandular portion, about 4.0 mm. long. The nerve ring surrounds the anterior portion at a point about a third of its length from the mouth opening, while the cervical papillae, which are small and inconspicuous, are slightly posterior to this. A number of strands of tissue leave the nerve ring and proceed to the cuticle where they are connected to depressed areas (Fig. 1) which are

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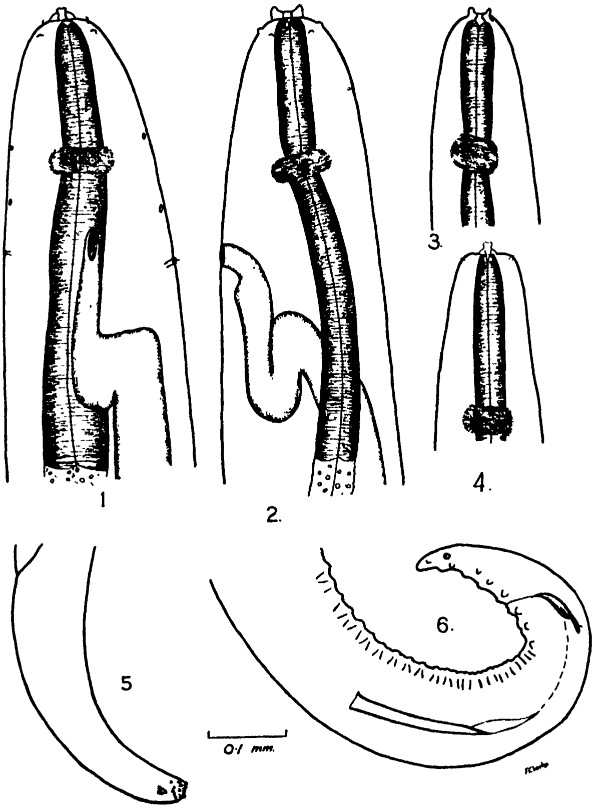
[2] *Research Professor of Parasitology, McGill University; and Director, Institute of Parasitology, Macdonald College, P.Q., Canada.*

possibly of a sensory nature. The female genital aperture is found close to these areas and they may function in connection with it.

The genital system is of the typical *Setaria* type.

The tail is slender and curved dorsally. It is 0.4 mm. long and terminates in a knob-like process covered with blunt spines. Two lateral, pointed appendices are found close to the tip (Fig. 5).



Setaria bidentata (Molin, 1858)

Fig. 1. *Head of female, ventral view.*
Fig. 2. *Head of female, lateral view.*
Fig. 3. *Head of male, lateral view.*
Fig. 4. *Head of male, ventral view.*
Fig. 5. *Tail of female.*
Fig. 6. *Tail of male.*

The male is smaller than the female and the tail end more tightly coiled. The two examples seen measured 33.75 mm. and 35.5 mm. respectively; the maximum thickness was 0.5 mm. The head end is similar to that seen in the female. The total length of the oesophagus is 0.35 mm.

The tail is bluntly pointed. There are four pairs of ventral post-anal papillae and a single pair of lateral post-anal papillae close to the tip. Only three pairs of pre-anal papillae were observed. From just anterior of the ano-genital opening to a point about 4.25 mm. from the tip of the tail, there are numerous *ventral* thickenings of the body, which under a low power of the microscope, superficially resemble papillae. The cuticle between these is transversely striated ventrally.

The two spicules are very dissimilar (Fig. 6). The smaller one is about 0.075 mm. long. The larger one is partly tubular and partly filamentous, the tubular part measuring 0.18 mm. long.

Three species of *Setaria* are known to occur in South American ruminants: *Setaria labiato-papillosa* (in cattle), *Setaria nudicauda* Ortlepp, 1924, from an unidentified member of the Cervidae in Dutch Guiana, and *Setaria bidentata* (Molin, 1858) from *Mazama nana* from Brazil. The present species differs from *S. labiato-papillosa* (which is practically cosmopolitan in its distribution) in a variety of points, especially in the shape of the peribuccal ring, and from *S. nudicauda*, in possessing a number of spines on the tail of the female.

Molin recorded *Filaria bidentata* from a closely related host in 1858. He described it as having the mouth armed with two teeth, both ends of the body tapering, the male tail rolled into many spirals, provided with numerous papillae ventrally and definitely pointed; the female tail loosely spiralled, its tip mucronate and spinose. The male measured up to 1¼ in. (32 mm.) and the female from 1¾ to 2¾ in. (44 to 70 mm.) in length.

This parasite does not appear to have been subsequently described. The original description is inadequate by modern standards but there is nothing in it which is incompatible with the description of the present species. Molin's reference to the numerous ventral papillae can quite well be interpreted as a reference to the true ventral papillae, together with the numerous ventral cuticular thickenings, which superficially resemble papillae. Moreover the hosts are very closely related zoologically. Accordingly, it is believed that the present species is the same as that seen in 1858 by Molin.

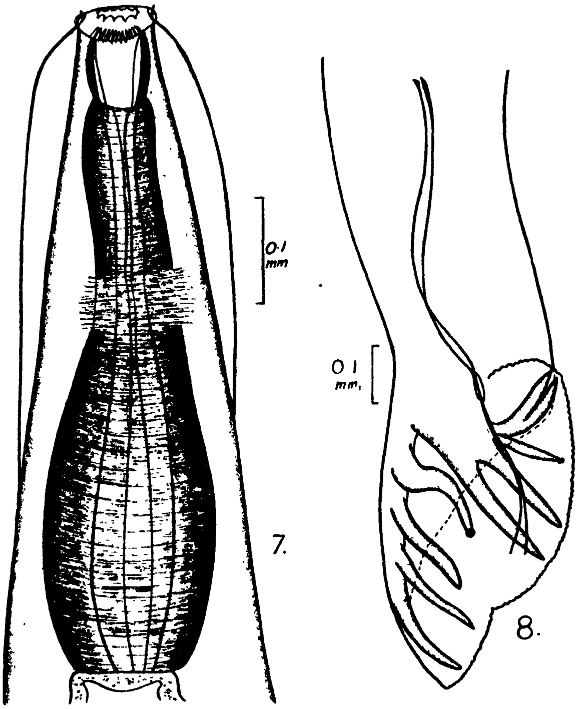
Eucyathostomum longesubulatum MOLIN, 1861

This parasite was recorded and described by Molin in 1861 from the caecum of the Pampas Deer *(Blastocerus campestris)* and the Red Brocket *(Mazama rufa)* from Brazil, but it does not appear to have been seen since. The writer recovered two males from the large intestine of *Mazama simplicicornis*.

The two males measure 10.5 mm. and 11.25 mm. respectively in length and 0.32 mm. in thickness. The cuticle is finely striated laterally with numerous longitudinal striations in the anterior region.

The mouth opening is terminal (Fig. 7) and communicates with a large, almost cylindrical buccal capsule, the inner surface of which carries a number of ridges, but no teeth. The writer was unable to identify a dorsal gutter. An internal leaf crown of some 28 pointed elements arises from the anterior edge of the buccal capsule. The mouth is surrounded by a ring of granular cuticle which is crenulated around the actual opening; this ring carries six circum-oral papillae.

The oesophagus is of the usual strongyle type and is 0.55 mm. long. The nerve ring is just anterior to the middle, but the small cervical papillae are situated almost at the level of the base of the oesophagus. The cuticle of the head region is swollen and the excretory pore is situated just at the posterior edge of this swelling.



Eucyathostomum longesubulatum Molin, 1861
FIG. 7. *Head of male.*
FIG. 8. *Bursa of male, lateral view.*

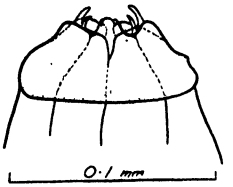
The genital system is of the usual strongyle type.

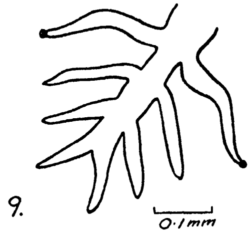
The bursa (Fig. 8) is voluminous and has a large dorsal lobe. The lateral and ventral rays arise from a common trunk. The ventral rays lie close together and are situated at right angles to the lateral rays. The lateral rays diverge slightly from each other, the externo-lateral being smaller and more slender than the others.

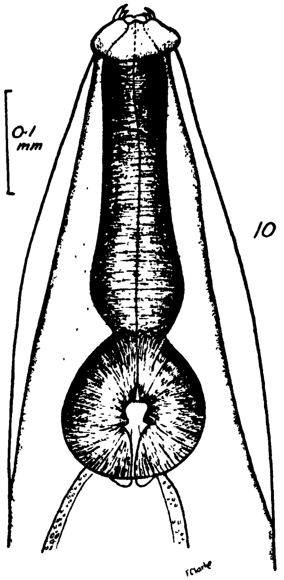
The dorsal ray is large and massive, the externo-dorsals arising some distance down its main stem. It is bifurcated distally (Fig. 9), each bifurcation being massive and simple. Two external branches are given off on each side of the dorsal ray between the externo-dorsal and the termino-dorsal rays.

The spicules are equal, long and filiform, measuring 0.9 mm. in length. Their free ends have a single lateral ala. Their points are barbed but are not fused together as in *Necator*. A small transparent accessory piece is present.

No females were found, but Molin describes these as 15 to 20 mm. long, with a long pointed tail. The vulva is situated just in front of the anus and has a distinct superior lip.







Eucyathostomum longesubulatum Molin, 1861.
FIG. 9. *Dorsal lobe of bursa.*

"Oxyuris" (s. l.) sp.
FIG. 10. *Head and oesophagus.*
FIG. 11. *Mouth opening.*

"Oxyuris" (s. l.) sp.

A single female oxyurid worm was also present in the large intestine, but owing to its immature condition and the absence of males, it is impossible to refer it to a genus.

It is 6.75 mm. long and has a maximum thickness of 0.3 mm. The cuticle is free from striations but the posterior end—especially the portion posterior to the anus—carries a number of small annular bands.

The mouth is surround by a granular cuticular collar (Figs. 10 and 11) carrying three fleshy lips and three small interlabia. Each lip has a long, transparent, medially curved process, medial to which is a smaller similar process. The interlabia are slightly trifurcated. The oesophagus is of the usual oxyurid type (Fig. 10), the anterior portion being 0.3 mm. long and the bulb 0.15 mm. in diameter. The cuticle in the oesophageal region is swollen. The excretory pore is situated posterior to the end of the oesophagus, 0.8 mm. from the anterior end of the body.

The vulva is at the junction of the middle and posterior thirds of the body, and the anus is 0.65 mm. from the tip of the sharply pointed tail.

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SCHISTOSOME DERMATITIS IN CANADA

NOTES ON TWO CAUSATIVE AGENTS AND THEIR SNAIL HOSTS IN MANITOBA[1]

By W. E Swales[2]

Abstract

An investigation of "swimmers' itch" at Clear Lake, Manitoba, revealed the presence of two schistosome cercariae, *Cercaria elvae* Miller, 1923 in *Lymnaea stagnalis jugularis* Say, and *Cercaria* sp. in *Stagnicola emarginata canadensis* (Sowerby); both species are known causes of the dermatitis. Five species of strigeid cercariae were found, none of which indicated any power of penetration into human skin. Copper sulphate, appropriately applied, and hand collection of adult snail hosts, are suggested for controlling schistosome dermatitis in Clear Lake.

"Swimmers' itch" or "slough itch" was first recognized as being caused by certain trematode cercariae by Cort (6) in Michigan. Since that time a certain amount of work has been done on the problem by workers in both Europe and North America. Several species of cercariae have been incriminated as causative agents, and until the work of McLeod (11) appeared they were all shown to be apharyngeal brevifurcate distome cercariae or typical members of the family Schistosomidae. However, McLeod recorded three species of cercariae from Clear Lake, Manitoba, all of which he described as penetrating human skin and causing a dermatitis. Two of these species he described as new to science and according to the illustrations they are members of the family Strigeidae and not Schistosomidae. The third is *Cercaria elvae* Miller, 1923, from a new snail host, *Lymnaea obrussa*.

In response to a request made by the National Parks Branch, Department of the Interior, the writer investigated the occurrence of the dermatitis at Clear Lake, Manitoba, in August, 1935. The following observations were made at Clear Lake and also upon material collected there and returned to the University of Michigan Biological Station and this Institute.

A survey of the beaches upon which the dermatitis has been recorded revealed the presence of a limited snail fauna as follows:—

Stagnicola emarginata canadensis (Sowerby) (Figs. 3–6); *Lymnaea stagnalis jugularis* Say. (Figs. 1–2); *Helisoma trivolvis* (Say); *Helisoma* sp.; *Helisoma* sp.; *Aplexa hypnorum tryoni* Currier; *Stagnicola palustris* var.; *Physella gyrina* (Say).

On the sandy beaches, which were almost devoid of macroscopic vegetation, the only snail in evidence was *S. emarginata canadensis*. This species was very common on the sand and stones at from three to seven feet below the surface

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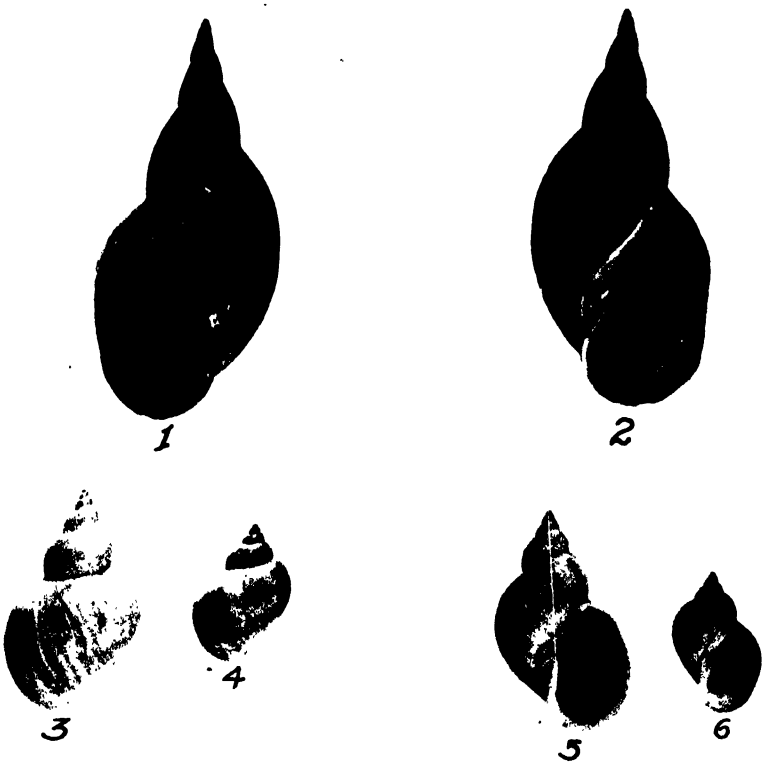
[2] *Research Assistant.*

PLATE I



FIGS. 1–2. *Lymnaea stagnalis jugularis Say, from Clear Lake, Manitoba.* (*Actual length —4 cm.*)

FIGS 3–6. *Stagnicola emarginata canadensis* (*Sowerby*) *from Clear Lake, Man.* (*Actual lengths 2.3 cm. and 1.4 cm.*)

of the water. In two small adjoining bays and in a creek (now dammed from the lake), which contained an abundance of macroscopic vegetation, the fauna included:—

L. stagnalis jugularis, *S. palustris* var., *A. hypnorum tryoni*, *P. gyrina*, and the *Helisoma* spp.

The bays also appeared to be the breeding places of *S. emarginata canadensis*, numerous young specimens of this species being present on the vegetation in shallow water.

Each snail collected was isolated in clear tap water and observed frequently over a period of 48 hours. If at the end of this period no cercariae had emerged, the specimen was crushed and examined microscopically. The results of these observations were noted and all infested snails which had shed cercariae were later taken alive to the Biological Station for further study of the cercariae. Still later, many specimens were collected from the beaches by co-operators and submitted to this Institute for further records.

The results of the field tests are as follows: Of 120 *S. emarginata canadensis* collected from the bathing beaches, seven shed schistosome cercariae alone, 24 shed strigeid cercariae alone, two shed both schistosome and strigeid cercariae, and one shed stylet cercariae. Of 272 specimens of *L. stagnalis jugularis*, collected from the creek and from one of the bays, 15 shed schistosome cercariae, 11 shed strigeid cercariae, and two shed both types. Of the other snails collected, *Helisoma trivolvis* was infested, two specimens of this species shedding a small straight-tailed strigeid, *Cercaria burti* Miller, 1923, in large numbers.

Almost every *S. palustris* var. tested shed echinostome cercariae or stylet cercariae, but no furcocercous forms.

Following a field study of the cercariae and of the ecology of the snail hosts, the living parasitized snails were taken to the University of Michigan Biological Station. The cercariae were there studied and comparisons were made with specimens from type or adjoining localities in Michigan.

The schistosome cercaria from *S. emarginata canadensis* was found to be identical with a schistosome cercaria from *S. emarginata angulata*. Dr. Benton S. Talbot, working at Douglas Lake, had recognized this species as being new to science and his description will be published in the near future. It must be referred to here as *Cercaria* sp. Twenty-one specimens of the former snail shed a single species of cercaria—that of *Diplostomum flexicaudum* (Cort and Brooks, 1928). The other three each shed a single species of strigeid cercariae, two having been previously recorded at Douglas Lake by Professor Cort, but as yet undescribed. The other was a strigeid of the Alaria type.

The two mixed infestations revealed the schistosome *Cercaria* sp. and *D. flexicaudum*.

The schistosome cercariae from *L. stagnalis jugularis* were all of a single species, *Cercaria elvae* Miller, 1923. All the strigeid cercariae found in this snail were also of a single species, *Diplostomum flexicaudum*.

In addition to the above, several specimens of *S. emarginata canadensis* contained tetracotyles of *Cotylurus flabelliformis*.

The snail fauna and the cercariae of Clear Lake do not differ materially from those of several lakes in the vicinity of the University Biological Station, Michigan. Differences noted were the scarcity of Physidae at Clear Lake and the apparent limitation of the dermatitis-producing schistosome cercariae to two species.

Owing to the fact that two species of strigeid cercariae had previously been reported as causing a dermatitis, each species of furcocercous cercaria collected was tested to determine its power of penetrating human skin. Three susceptible volunteers were first used, and later four more were used for comparison. The cercariae were carefully isolated under a binocular microscope to avoid confusion due to mixed infestations.

In every case *Cercaria elvae* from *L. stagnalis jugularis* caused lesions typical of schistosome dermatitis. In four subjects the schistosome *Cercaria* sp. from *S. emarginata canadensis* caused similar lesions. The writer is very susceptible to the dermatitis and the sensations experienced on several experimental exposures were similar to those described by other workers. As the water containing the cercariae dries on the skin a prickling sensation is felt. This subsides in from five to ten minutes, leaving no visible lesion. During the night following the day of exposure an intense skin irritation occurs and the next day red papules, 5–15 mm. in diameter, are present. In my case these papules, unless scratched, disappeared by the third day, and the irritation was noticed only during the first 24 hours after exposure. No cercariae other than those mentioned gave any indication of penetrating skin, even although they were placed on arms and legs in large numbers and the water was allowed to evaporate.

Discussion of the Observations

Clear Lake, in common with similar lakes in the mid-western United States, has been found to be a habitat of two important gastropods, varieties of *S. emarginata* and *L. stagnalis*. Both of these snails are intermediate hosts of schistosome trematodes, the cercariae of which are capable of penetrating human skin and causing a temporary dermatitis. Owing to the lack of macroscopic vegetation and the sandy and stony bottoms of the beaches, *S. emarginata* is the common snail on the areas used for bathing by visitors. *L. stagnalis* is limited in its distribution near the beaches to small bays and a creek which contain an abundance of vegetation. Thus *Cercaria elvae* is of secondary importance as a causative agent of dermatitis, whereas the schistosome *Cercaria* sp. from *S. emarginata* is actually present on the bathing beaches whenever conditions are favorable for its emergence. Of the two snail hosts *L. stagnalis jugularis* can be destroyed by means of copper sulphate broadcast over the shallow water habitats. In controlling *S. emarginata canadensis* the methods must be such that the lethal agent will diffuse over the surface of the sand and stones in deeper water. Collection by hand of the adult snails is also indicated as a means of reducing their numbers.

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The most common cercaria in Clear Lake, that of *D. flexicaudum*, is a strigeid, and does not cause the dermatitis. It is, however, very pathogenic to fish, penetrating the eyes and causing intense inflammatory processes, which, in tests conducted with Clear Lake material, were followed by death in 48 to 72 hours. Young *Ameiurus nebulosus* were found to have the cercariae actively moving around the lens of the eyes after the fish's death. As pointed out by Van Haitsma (16) and others, this cercaria might have an important bearing on the health of fresh-water fishes.

The cercaria of *D. flexicaudum* is the strigeid found in mixed infestations with schistosome cercariae, in both snail hosts in Clear Lake. This fact might lead to confusion in certain cases.

The writer's findings must be compared with those of McLeod who worked on the same area in 1933. McLeod (11) found one schistosome and two strigeid species in *Lymnaea obrussa*. This snail (now *Fossaria obrussa* (Say) Baker, 1928) according to Baker (1, 2) is found generally in small bodies of water, such as creeks, ponds, sloughs, bays and marshy spots along river banks. It frequently inhabits abandoned quarries which have become filled with water. Like *Fossaria modicella* and *F. parva*, this species is at home on sticks, stones and exposed muddy surfaces. Although the writer has frequently collected *F. obrussa* in other parts of Canada he was unable to find it on the shore of Clear Lake. It seems possible that McLeod was working with *Stagnicola emarginata canadensis*. In any case the occurrence of *Cercaria elvae* in either of these snails needs further study.

Cercaria bajkovi McLeod, 1934, was not found during this investigatio but a continued search might have revealed this species. In the search fo *C. wardlei* McLeod, 1934, each lot of the peculiar flexed-tail forms was carefully studied, without result. In each case these forms were the cercariae of *D. flexicaudum*, which according to McLeod (11) differ from *C. wardlei* in th number of penetration glands, the caudal flame-cell pattern and in the absenc of oral bristles in the latter species.

Owing to the great prevalence of strigeid cercariae in all fresh water McLeod's record of the penetration of mammalian skin by members of this group is important and needs further study.

Acknowledgments

I wish to express great appreciation of the help and co-operation of the National Parks Branch of the Department of the Interior of Canada. Mr. J. Smart, Park Superintendent, who laid the foundation for investigational work on this subject at Clear Lake, and who is now applying measures of control, provided invaluable assistance. Dr. G. Stevens, Parks Physician, also helped materially.

To Drs. Geo. LaRue, W. W. Cort and Benton Talbot, I am deeply indebted for the opportunity to work at Douglas Lake and for invaluable advice, direction, and confirmation of identifications. Mr. A, La Rocque, Department of Mines, Ottawa, kindly identified the snail hosts.

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BUNODERINA EUCALIAE GEN. ET SP. NOV., A NEW PAPILLOSE ALLOCREADIIDAE FROM THE STICKLEBACK[1]

BY M. J. MILLER[2]

Abstract

A new species of trematode is described from the intestine of the stickleback *(Eucalia inconstans)*, and referred to a new genus.

In the last week of October and in early November, 1935, a number of brook sticklebacks (*Eucalia inconstans*) were examined and found to harbor a trematode, the consequent study of which showed it to be, as yet, undescribed. This fluke belongs to the family Allocreadiidae, and more specifically to that group known as the papillose Allocreadiidae. The characteristics revealed by this trematode necessitate the formation of a new genus for which the name *Bunoderina* is proposed, and for the species, *B. eucaliae* is proposed.

The specimens studied were fixed in Schaudinn's solution, stained in alum carmine and mounted in balsam. It was found that specimens in tap water passed out their eggs; these specimens were useful for determining the form of the uterus, but could not be satisfactorily fixed. Considerable difficulty was encountered in fixing the trematodes in a normal position. Satisfactory results were obtained by placing the entire intestine of the fish in hot fixative, and then dissecting out the trematodes.

This species is found attached by its suckers or free in the intestine of the sticklebacks.

Specific Diagnosis

Body thick and muscular, almost circular in cross section, 0.83 to 1.06 mm. long and 0.31 to 0.35 mm. wide. One sexually immature specimen measured 0.56 mm. in length. Body widest at the middle and narrowing off at both ends. Cuticle smooth. Suckers comparatively large, ventral sucker larger than the oral sucker. Pharynx well developed. Oesophagus long, S-shaped unless the anterior end of body is extended; it bifurcates at a point slightly anterior to the acetabulum. Intestinal crura wide and contractile, extending down into the third quarter of the body, dorsal to and slightly overlapping the vitellaria.

[1] *Manuscript received February 3, 1936.*
Contribution from the Institute of Parasitology, McGill University, Macdonald College, Que., with financial assistance from the National Research Council of Canada.

[2] *Graduate Student.*

Testes sub-circular, usually oblique but may be side by side, situated in the third quarter of the body. Vasa efferentia enter the cirrus sac separately. Cirrus sac small and relatively broad, overlapping the ventral sucker to about one-third its diameter; it contains a seminal vesicle and a cirrus. Finer morphology of cirrus sac was not studied.

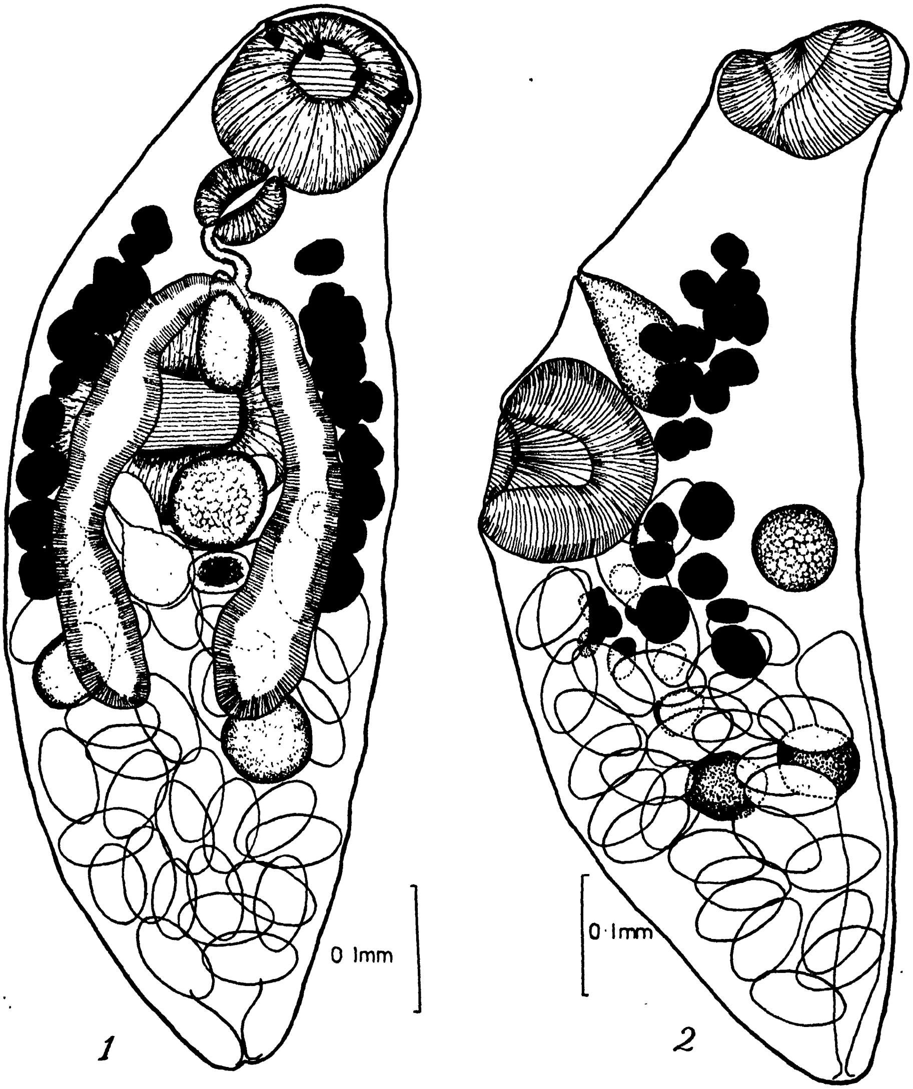

FIG. 1. *Bunoderina eucaliae gen. et sp. nov. 1. Dorsal view. 2. Lateral view.*

Ovary circular, dorsal to the posterior part of the acetabulum. Spermatheca large, directly caudad of the ovary. Laurer's canal with bulbous base. An oötype or shell gland could not be detected. Uterus a convoluted tube filling the posterior part of the body. Vitellaria composed of comparatively few, large follicles, extending from a short distance anterior of the acetabulum to the posterior portion of the middle third of the body, terminating just anterior of the end of the intestinal crura.

Excretory vesicle a bladder-like structure which may extend forward as far as the acetabulum.

Eggs ovoid, 0.07 to 0.83 mm. long by 0.044 to 0.047 mm. wide, light brown in color and containing active miracidia when deposited.

Host: Brook stickleback (*Eucalia inconstans*).

Location: Small intestine.

Locality: Drainage canal, Jacques Cartier Co., Que., Canada.

Discussion

The description of *Bunoderina eucaliae* as given agrees with all the specimens examined. There is, however, a variation in shape of body that corresponds with age. In the older trematodes the posterior part of the body becomes wider with the increasing number of eggs; it does not however, become sacculate as in *Bunodera sacculata.*

B. eucaliae differs from other species of the papillose Allocreadiidae in the form of the uterus and the reduced number of vitelline follicles. As has been stated, its uterus is a narrow convoluted tube, filling the posterior portion of the body. Nowhere else in the papillose Allocreadiidae does this type of uterus occur. In *Crepidostomum* and *Megalogonia* the uterus is a narrow tube, but it is characteristically short, with few, if any, convolutions. *Bunodera* has the ascending ramus of the uterus expanding into a uterine sac. The writer therefore considers the form of the uterus to be a character of generic importance, and thinks it justifiable to create a new genus with *B. eucaliae* as the type and only species.

Generic Diagnosis

Allocreadiidae, with six oral papillae, four dorsal and two ventro-lateral. Ventral sucker larger than the oral sucker. Testes sub-circular, obliquely arranged to nearly opposite, situated in the third quarter of the body. Uterus a narrow convoluted tube, filling the posterior part of the body. Vitelline follicles reduced in number.

Holotype Specimen in Helminthological collection, Institute of Parasitology, McGill University, Macdonald College, P.Q., Canada.

Paratype deposited at Helminthological collection, Department of Zoology, University of Illinois, Urbana, Illinois, U.S.A.

Acknowledgment

It is with great pleasure that I express my indebtedness to the Director and staff of the Institute of Parasitology, McGill University, for their very helpful advice and assistance. I wish also to thank Dr. H. J. Van Cleave, of the University of Illinois, for supplying a specimen of *Bunodera sacculata.*

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A GRAPHICAL METHOD FOR THE RAPID ESTIMATION OF THE STANDARD DEVIATION[1]

By R. Holcomb[2] and Darol K. Froman[3]

Abstract

A graphical method, based upon Galton's "ogive" curve, that may be used for estimating the various criteria of variability including the standard deviation, is described. Curves of this type are more readily adaptable to tests for normality of distribution than are the customary frequency histograms, especially with a limited number of observational data.

The purpose of this paper is to present a simple graphical method for the estimation of standard deviation and the degree of normality of a distribution. This method may be found useful by those who have not available the facilities of a statistical laboratory. It is essentially a short-cut, facilitating the analysis of observational data with special reference to the variation within a group, as measured by the standard deviation and the probable errors of averages. The same graphical method may also serve to illustrate variation, both absolute and relative, as when applied either to one or to more groups of observations.

It is currently popular to illustrate variation by means of frequency histograms. For such diagrams, it is necessary to group the data, and if the number of observations is small, either the variation within a group is so large, or the number of groups is so small, that it is difficult to estimate whether the distribution approaches normality. In this case estimates of such quantities as standard deviation cannot be made from the graph. Even for large numbers of observations little more may be obtained from the diagram than the position of the mode, the two extremes, and the probable position of the arithmetic mean.

The graphical method given here can be described best by means of an example. Table I gives the percentage of fat contained in the thigh muscles of 139 chickens. The data are arranged in order of increasing percentage and numbered in order from 1 to 139. If then, the percentage of fat be plotted against the number of the observation, we obtain the distribution shown in Fig. 2. Curves of this form were first proposed by Sir Francis Galton (1) in 1875, but little use has since been made of them. Since each observation appears as a plotted point, the form of the distribution is made directly evident, even for a small number of observations where a frequency histogram might be quite useless. As Galton pointed out, the median, the quartiles and the deciles may be read directly from this graph. All of these points are of value in describing the distribution of the observed values.

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[1] *Manuscript received September 25, 1935.*
Contribution from the Faculty of Agriculture of McGill University, Macdonald College, Que., Canada. Macdonald College Journal Series No. 71.

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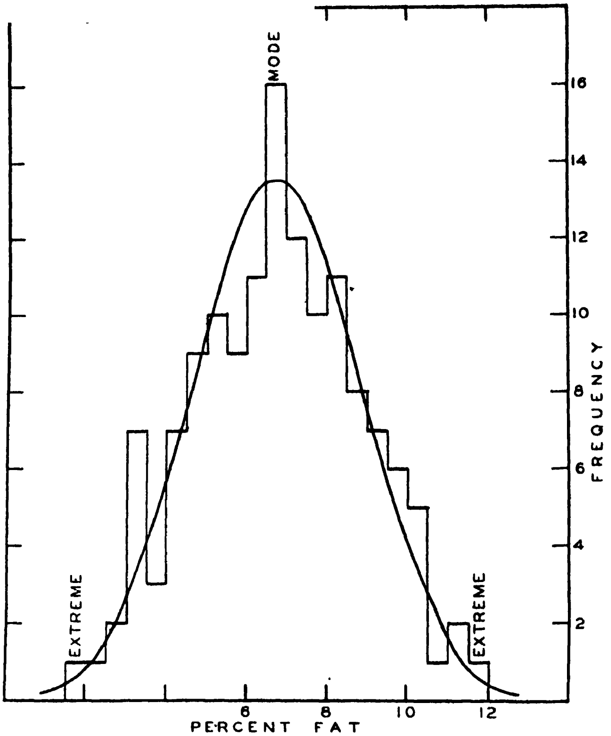

FIG. 1. *Frequency histogram showing distribution of the fat content of thigh muscles of the domestic fowl (Cf. Fig. 2 showing the same data arranged as an ogive curve).*

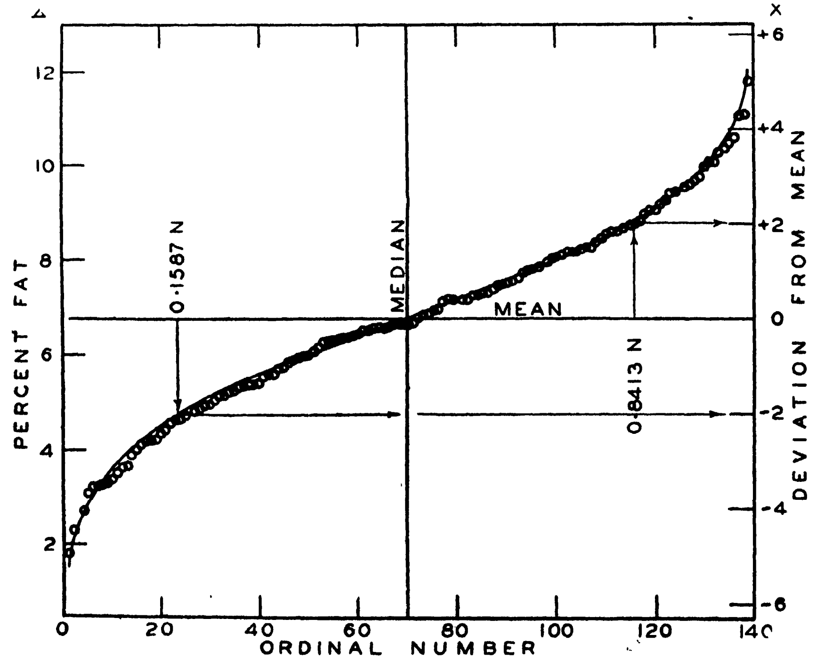

FIG. 2. *Graphical approximation of the standard deviation from an ogive curve.*

TABLE I

THE FAT CONTENT OF THE THIGH MUSCLES OF CHICKENS

No.	% fat	No.	% fat	No.	% fat	No.	% fat
1	1.86	36	5.33	71	6.69	106	8.24
	2.36		5.34		6.78		8.26
	2.58		5.37		6.85		8.38
	2.76		5.38		6.86		8.42
5	3.12	40	5.41	75	6.92	110	8.56
	3.25		5.57		6.99		8.59
	3.27		5.61		7.12		8.61
	3.31		5.61		7.14		8.65
	3.32		5.75		7.14		8.70
10	3.42	45	5.76	80	7.14	115	8.72
	3.45		5.85		7.15		8.77
	3.63		5.92		7.15		8.83
	3.68		5.96		7.23		9.00
	3.92		5.97		7.25		9.05
15	4.03	50	6.01	85	7.29	120	9.07
	4.15		6.18		7.30		9.16
	4.21		6.29		7.37		9.26
	4.21		6.32		7.43		9.40
	4.23		6.33		7.50		9.40
20	4.39	55	6.34	90	7.50	125	9.50
	4.47		6.38		7.58		9.51
	4.60		6.39		7.61		9.56
	4.67		6.42		7.72		9.65
	4.67		6.44		7.77		9.71
25	4.68	60	6.47	95	7.79	130	9.98
	4.79		6.51		7.81		10.03
	4.85		6.52		7.89		10.05
	4.87		6.56		7.93		10.23
	4.95		6.57		8.02		10.31
30	4.98	65	6.57	100	8.09	135	10.43
	5.06		6.58		8.10		10 51
	5.16		6.64		8.13		11.01
	5.17		6.64		8.13		11.03
	5.22		6.65		8.17	139	11.79
35	5.24	70	6.66	105	8.19		

By use of the deciles the arithmetic average may be rapidly approximated, for it is evident that these deciles should be regularly spaced about the average, if the distribution is normal. The differences of the deciles from the median are averaged (Table II) for the corresponding pairs, and a set of corrected "medians" obtained by adding these averages to the deciles. The grand average of these corrected "medians" is then taken as the average of the entire group. For the data used here, the estimated average is found to be 6.75, which agrees perfectly with the calculated arithmetic average.

In Fig. 2, the mean value for the percentage of fat may now be drawn, and the scale *(X)* may now be changed to read deviations from the mean (*x*-scale). It will be observed that the points in Fig. 2 appear to follow a

TABLE II

THE APPROXIMATION OF THE ARITHMETIC AVERAGE BY THE USE OF DECILES

Decile number	Observ. number	Decile value	Differences from median			
1	14	3.92				2.74
2	28	4.87			1.79	
3	42	5.61		1.05		
4	56	6.38	0.28			
5	70	6.66				
6	84	7.25	0.59			
7	98	7.93		1.27		
8	112	8.61			1.95	
9	126	9.51				2.85
Sums			0.87	2.32	3.74	5.59
Mean			0.43	1.16	1.92	2.80
Corrected median			6.81	6.77	6.73	6.71
Grand average			6.75			

smooth curve, starting steeply, gradually flattening out across the centre, and rising steeply again towards the end. It can be shown that, for a perfectly normal distribution, the relation which fits such an arrangement is given by the equation,

$$n = \frac{N}{2} \pm \frac{N}{2\sqrt{\pi}} \int_{0}^{\frac{x}{\sqrt{2}\sigma}} e^{-\frac{x}{2\sigma^2}} \,.\, d\left(\frac{x}{\sqrt{2}\sigma}\right)$$

where N is the total number of observations, σ is the standard deviation, and x is the deviation from the mean of the n^{th} observation. The number n is the serial number of the observation in the arranged order.

The curve for the data illustrated here, where N is equal to 139 and σ, calculated from residuals, is equal to 2.06, has been superimposed upon the points. It appears to fit the data remarkably well.

The general properties of curves of this family are shown in Fig. 3, where six curves are given for values of σ from 1 to 6. When σ is equal to zero, or no variation is shown in the group, all values would lie along the mean line. As the degree of variation becomes larger or σ increases, the curves cross the mean line at greater and greater angles, at the same time becoming more nearly straight lines. A special point of interest is that at any point along the N-axis, the distance of the curve from this axis is directly proportional to the standard deviation. As shown in Fig. 3, the ratios of the distances of the curves from the N-axis are as 1 : 2 : 3 : 4 : 5 : 6. This leads directly to the method for the approximation of the standard deviation.

For example, when,

$$x/\sigma = 0.5, \text{ then } n = N/2 \pm 0.1918N,$$
$$x/\sigma = 1.0, \text{ then } n = N/2 \pm 0.3413N,$$
$$\text{and } x/\sigma = 1.5, \text{ then } n = N/2 \pm 0.4332N.$$

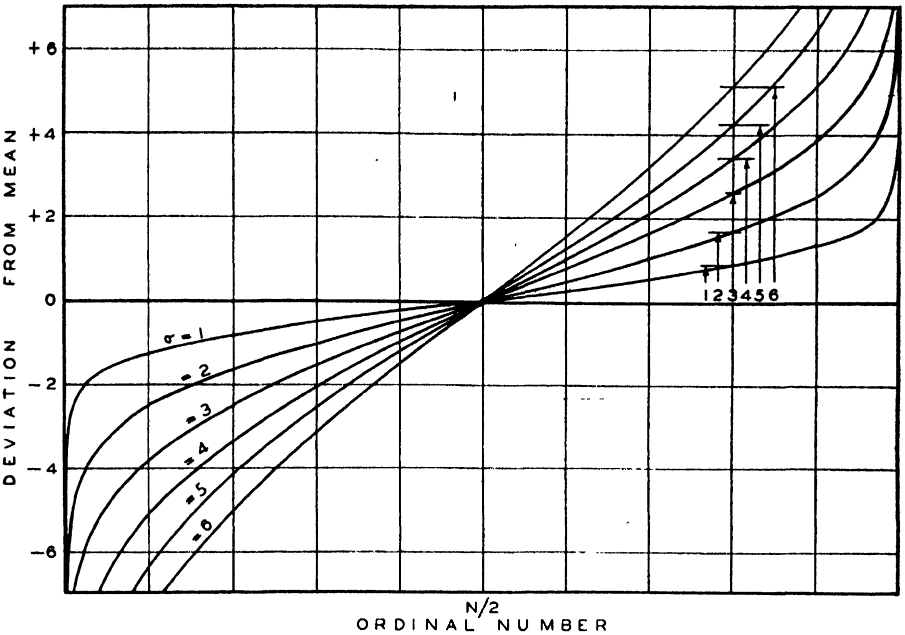

FIG. 3. *Family of ogive curves for several standard deviations.*

All that is required is to read from the graph the values of x corresponding to the six values of n given in the right-hand equations above. Six approximations of σ are obtained, the average of which is assumed to be a good estimate. The detailed work is given in Table III.

The method is shown graphically in Fig. 2. If the curve is fairly symmetrical about the mean and median, a fair estimate of the value of σ can be obtained by reading only two values of x at $n = N/2 \pm 0.3413N$. The accuracy of the estimate is improved, in general, by increasing the number of approximations. Perpendiculars have been erected from the points $0.1587N$ (22) and $0.8413N$

TABLE III

THE APPROXIMATION OF THE STANDARD DEVIATION

x/σ	n	x	σ
0.5	$0.3083N$ (42.9)	1.14	2.28
0.5	$0.6917N$ (96.1)	1.07	2.14
1.0	$0.1587N$ (22.0)	2.15	2.15
1.0	$0.8413N$ (117)	2.07	2.07
1.5	$0.0668N$ (9.3)	3.40	2.26
1.5	$0.9332N$ (130)	3.15	2.10
Average			2.16
Calculated			2.06
Error			5%

TABLE IV

VALUES OF THE PROBABILITY INTEGRAL FOR RATIOS OF x/σ*

x/σ	$I=\frac{1}{2\sqrt{\pi}}\int_0^{\frac{x}{\sqrt{2}\sigma}} e^{-\frac{x^2}{2\sigma^2}} .d\left(\frac{x}{\sqrt{2}\sigma}\right)$	$\log_{10} I$
0.25	0.0988	$\bar{2}.99476$
0.50	0.1917	$\bar{1}.28262$
0.75	0.2732	$\bar{1}.43648$
1.0	0.3413	$\bar{1}.53314$
1.5	0.4332	$\bar{1}.63669$
2.0	0.4772	$\bar{1}.67870$
2.5	0.4937	$\bar{1}.69346$
3.0	0.4986	$\bar{1}.69801$
4.0	0.4999	$\bar{1}.69888$

For table from x/σ = 0.00 to 4.24 by units of 0.01 see Medical Biometrics, by Raymond Pearl, Philadelphia, 1923.

(117). The point of intersections, when carried over to the x-scale, gives the value of the standard deviation, since at these two values of n, x/σ is equal to 1.0.

In Table IV the values of the integral are given for various values of the ratio x/σ from which curves to fit any data may be rapidly computed. The data for the fitting of the curve in Fig. 2 is given in Table V.

TABLE V

DETAILS OF CURVE-FITTING FOR FIG. 2

x/σ	$\frac{1}{2\sqrt{\pi}}\int_0^{\frac{x}{\sqrt{2}\sigma}} e^{-\frac{x^2}{2\sigma^2}}.d\left(\frac{x}{\sqrt{2}\sigma}\right)$	Integral times N	$+N/2$	$-N/2$	x
0.25	0.0988	13.7	83.2	55 8	0.51
0.50	0.1917	26.6	96.1	42.9	1.03
0.75	0.2723	38.0	107.5	31.5	1.54
1.0	0.3413	47.4	116.9	22.1	2.06
1.5	0.4332	60.2	129.7	9.3	2.09
2.0	0.4772	66.4	135.9	3.1	4.12
2.5	0.4937	68.6	138.1	0.9	5.15

The value of the method lies in the ease with which an approximation to σ may be obtained from an immediate plot of the data, and in the usefulness of such a graph for judging the skewness of the deviations for small numbers of observations. With very little practice it is possible to tell from such curves whether the distribution approaches normality sufficiently to apply a more rigorous statistical treatment.

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VITAMIN A REQUIREMENTS OF GROWING CHICKS

I. PRELIMINARY STUDIES WITH "REFERENCE" COD LIVER OIL[1]

BY JACOB BIELY[2] AND WILLIAM CHALMERS[3]

Abstract

In experiments conducted to determine the requirements of chicks for vitamin A up to eight weeks of age, vitamin A was supplied, in addition to the basal ration, by oral administration of Reference Cod Liver Oil at the rate of 25, 50, 75 and 100 International units per day. It was found that chicks receiving 75 units of vitamin A per day grew at a normal rate and did not show any signs of vitamin A deficiency. The chicks that received the basal ration only, showed symptoms of avitaminosis A at three weeks of age, while the group receiving 25 international units per day at eight weeks clearly had not received a sufficient supply of the growth factor.

Although the role of vitamin A in promoting normal growth and development of fowls was established over a decade ago (5), comparatively little is known about the quantitative requirements of growing chicks for this dietary factor. This note reports preliminary results obtained in feeding chicks graded quantities of vitamin A, using the International unit as the basis of measurement. Reference Cod Liver Oil (vitamin A) obtained from the Committee of Revision of the Pharmacopoeia of the United States of America (2) was used for this purpose. This oil is standardized to contain 3000 international units of vitamin A in one gram of the oil. The vitamin D content of the oil is not specified. In order to ensure an ample supply of vitamin D the ration was supplemented with irradiated yeast.

Experimental Methods

Day-old sexed S.C. White Leghorn cockerels were divided into five groups of 12 chicks each, except the control, which consisted of 21 chicks. They were placed in a battery brooder and fed when 24 hours old. Each tier of the battery brooder was 24 × 60 in. and divided into two compartments of equal size. One of the compartments in each tier was heated by three carbon filament lamps.

The chicks were fed a basal ration similar to that used by Elvehjem and Neu (4) in vitamin A determinations, except that irradiated yeast (70 D) served as a source of vitamin D instead of ultra-violet light. This ration was thought to be free of vitamin A, but in the light of the recent work of Giddings and Swim

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Contribution from the Department of Poultry Husbandry, The University of British Columbia, Vancouver, Canada.

[2] *Instructor, Department of Poultry Husbandry, The University of British Columbia.*

[3] *Western Chemical Industries, Ltd., Vancouver, Canada.*

(6) on the vitamin A content of casein and yeast, it would appear that it contained traces of the vitamin. The basal ration and water were continually available to the birds. Analyses of the tibia of several control chicks showed that bone calcification was normal, the ash content being over 45%.

Four groups of chicks were given supplements of vitamin A once a week at the respective rates of 25, 50, 75 and 100 International units per day. By dilution with the calculated proportions of Wesson oil, the Reference Cod Liver Oil was made up into standard solutions containing the seven-day quotas of 175, 350, 525, and 700 international units per cubic centimetre. At the beginning of each week these solutions were fed directly into the crop by pipette in 1 cc. amounts. That the feeding of vitamin A once a week gives results comparable to daily administration has been shown by the work of Coward and Key (3) on rats.

The control chicks were fed the basal ration only.

Results

The average weekly weights of the chicks in the various groups are shown in detail in Table I, and graphically illustrated in Fig. I.

TABLE I

AVERAGE WEEKLY WEIGHTS OF ♂ CHICKS

Lot No	Number of International units of vitamin A fed per day	Average weights								
		1 day old gm	1 week gm	2 weeks gm	3 weeks gm	4 weeks gm	5 weeks gm	6 weeks gm	7 weeks gm	8 weeks gm
1	100	36.2	59.3	98.5	139.6	204.8	282.2	359.0	446.4	543.4
2	75	36.0	58.2	91.3	133.6	197.0	268.2	348.2	428.2	552.4
3	50	37.3	62.7	100.9	143.2	194.4	257.0	329.3	427.5	507.7
4	25	38.1	62.7	101.0	140.6	191.2	253.0	316.7	381.7	421.2
5	Control	34.8	58.1	87.1	115.9	151.3	176.8	209.3	387.0*	All dead

**2 chicks only*

The chicks in the control group began to show definite symptoms of avitaminosis A at three weeks of age. The growth rates of the control chicks are in accord with those reported by Elvehjem and Neu (4). The chicks that received the basal ration only, in all cases showed loss in weight* shortly before death. Post-mortem examination invariably revealed the presence of urates in the kidneys and ureters. Lesions in other organs were not observed as frequently as in the kidneys or ureters. The first mortality occurred at three weeks of age and the last during the eighth week. All the chicks in this group showed marked symptoms of vitamin A deficiency at five weeks of age. The ability of some of them to live beyond that period was probably due to individual reserves and the trace of vitamin A in the ration, apparently sufficient to support subnormal life.

The chicks receiving graded quantities of vitamin A grew at a fairly uniform rate up to four weeks of age. After the end of the fourth week the chicks

**The weights of the chicks that died during the week are not included amongst the average weekly weights shown in Table I and Fig 1.*

which received 25 units of vitamin A began to lag in growth, so that by t[illegible] of the eighth week they weighed significantly less than the chicks re[illegible] 50 International units. Similarly, the chicks which received 50 uni[illegible] at a somewhat slower rate than the ones which received 75 units, a[illegible]

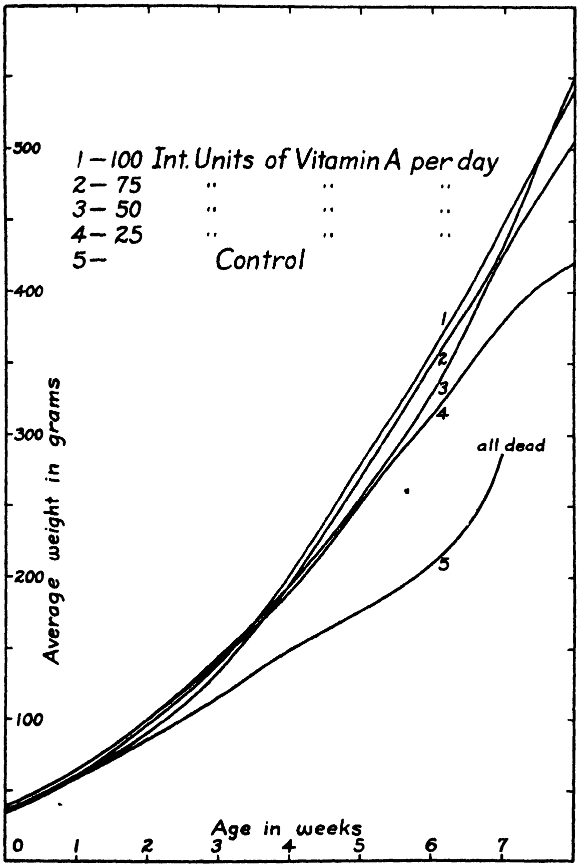

FIG. 1. *Growth curves of chicks which received various supplements of vitamin A.*

chicks which received 75 units grew at a slightly slower rate than the chic[illegible] which received 100 units. For some reason unaccounted for, those receivin[illegible] 100 units of vitamin A did not show corresponding gains during the eigh[illegible]h week. A statistical analysis of the weights of the individual chicks at eig[illegible] weeks of age is given in Table II. Table III shows the significance of t[illegible] differences of the average weights of the groups at eight weeks of age.

TABLE II

[illegible]TISTICAL ANALYSIS OF THE WEIGHTS OF CHICKS AT EIGHT WEEKS OF AGE

[illegible]	Mean weight	Standard deviation	Coeff. of v[illegible]
25 [illegible]	421.2 ± 22.93	96.16 ± 16.21	22.83 ± [illegible]
50 Int. [illegible]	507.7 ± 22.28	93.44 ± 15.76	18.41 ± [illegible]
75 Int. un[illegible]	[illegible] ± 16.23	68.05 ± 11.48	12.32 ± [illegible]
100 Int. units/day	[illegible] ± 20.87	97.87 ± 14.76	18.01 ± [illegible]

TABLE III

STATISTICAL SIGNIFICANCE OF DIFFERENCES BETWEEN WEIGHTS AT EIGHT WEEKS OF AGE

Supplements	Difference of means	Difference / Probable error	Significance of the difference
25 units: 50 units/day	86 5 ± 31 97	2 706	Probably significant
50 units: 75 units/day	44 7 ± 27 56	1 622	Not significant
75 units: 100 units/day	9 0 ± 26 44	0 3403	Not significant
25 units: 75 units/day	131 2 ± 28 09	4 671	Significant

With the exception of the chicks which received 25 units, none of the groups showed any symptoms of avitaminosis A at eight weeks of age. As a matter of fact, the chicks appeared to be well developed, well feathered out and were apparently normal. Those which received 75 and 100 units per day compared favorably in weight with the average weight reported by Buckner *et al.* (1) for chicks brooded in confinement. It is of interest to note that the average weights of the chicks receiving 75 to 100 units of vitamin A equalled those receiving an otherwise identical ration, used in vitamin D assays, in which yellow corn was substituted for the white corn (59%). A repetition of the experiment at a later date gave practically identical results.

Conclusions

In an experiment of this nature where the amount of vitamin A supplied to each individual is constant, a period of eight weeks is apparently long enough to differentiate the growth responses of the chicks fed 25 units from those fed the larger unitages.

Had the oil been added to the mash in different percentages, the difference in the growth of the several lots of chicks would not have become so distinctly evident, since the greater the consumption of feed, the greater correspondingly the amount of vitamin A consumed. It may be concluded that the administration of 75 international units of vitamin A per day would ensure normal growth and protect chicks up to eight weeks against any symptoms of vitamin A deficiency. With the rate of growth obtained in this experiment, 100 units of vitamin A per day would appear to be above the immediate normal requirements of chicks up to two months of age.

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STUDIES ON THE ENDOPARASITIC FAUNA OF TRINIDAD

III. SOME PARASITES OF TRINIDAD CARNIVORES[1]

BY THOMAS W. M. CAMERON[2]

Abstract

Two new species of cestodes, five of nematodes, two of Acanthocephala and one linguatid are recorded from carnivores in Trinidad, B.W.I.

During 1935, the writer received from Professor F. W. Urich, Trinidad, B.W.I., the entrails of an ocelot or tiger-cat *(Felis pardalis)*, four "mangrove dogs" or crab-eating racoons *(Procyon cancrivora)* and three "wood-dogs" or Tayras *(Tayra barbara)*. The only other carnivore known to exist in Trinidad, is the very rare otter *(Lutra insularis)*, and so far it has not been possible to secure a specimen. All of the eight animals received contained internal parasites. These consisted of two species of cestodes, five of nematodes, two of acanthocephala and one linguatid; no trematodes were found.

In addition to parasites, the stomachs and intestines of the racoons contained many remains of beetles and crabs, while in the ocelot were parts of an agouti as well as two decomposed trichostrongyles of a species found in that host: these accordingly, are not recorded as parasites of the ocelot.

I have to acknowledge the assistance both of Professor R. T. Leiper of the Imperial Bureau of Agricultural Parasitology and Dr. M. C. Hall of the United States Bureau of Animal Industry, in obtaining both references and literature. I have also to acknowledge the willing assistance of the Division of Research Information of the National Research Council of Canada in the translation of a number of papers.

Cestoda

Two—possibly three—species of cestodes, all belonging to the genus *Diphyllobothrium*, were recovered from the intestines of Trinidad carnivores. One species was found in the ocelot, and one in the racoon: the third species was also found in the racoon in very large numbers and may merely represent the young stages of the second species. In order, however, to avoid subsequent confusion, it is described merely as *Diphyllobothrium* sp.

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Contribution from the Institute of Parasitology, McGill University, Macdonald College, P.Q., with financial assistance from the National Research Council of Canada.

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Diphyllobothrium urichi sp. nov.

Several specimens of this species were recovered from the small intestine of the ocelot *(Felis pardalis).* They had been fixed *in situ* in formalin, and the process of opening the intestine unfortunately prevented any complete tapeworms being recovered. The total length cannot, therefore, be given, but several pieces more than 20 cm. long were recovered, and it is probable that specimens reached at least 30 cm. in length.

A single head was seen (Fig. 1.). It was almost as long as it was broad. The bothria are conspicuous and have free anterior margins; the posterior margins gradually join the neck. The anterior end of the scolex projects beyond the bothria, which appear to be situated laterally; owing to twisting of the neck, this point requires confirmation. The neck is long but broad and the whole appearance of the anterior end of the body is quite different from that of *D. latum.*

The mature segments are shown in Figs. 2 and 3, the latter being an older one towards the end of the chain. The oldest segments are approximately square: the younger ones are broader than long. None is longer than broad.

The male and female openings are on the mid-ventral line, almost a quarter of the length of the segment from its anterior margin. The uterine pore is almost at the centre point of the segment but is always slightly to one side of the mid-ventral line.

The uterus is convoluted, being thrown into four or five small lateral loops. It passes for a short distance in front of the uterine pore but shows no tendency to form a "rosette." It always passes posterior to the ovarian commissure and, in fully mature segments, always makes an indentation in the posterior margin.

The ovary is quite typical. The yolk follicules and testes are very numerous and almost equal in size. They completely fill both sides of the segment and meet in the middle line, anterior to the genital pore. Both testes and yolk follicules are completely absent from an oval space around the genital tubes and they do not meet posterior to the ovary.

The eggs are oval and measure 50μ long by 30μ broad.

In this species, the uterus is confined to the medial region of the posterior half of the segment, and a post-ovarian portion, in fully mature segments, always projects into the succeeding segments. The genital pore opens about a third of the distance from the anterior border and both testes and yolk glands are continuous across the middle line, anterior to the cirrus sac, but *not* posterior to the ovary.

Its closest relation would appear to be *D. decipiens* (Diesing) as described by Faust, Campbell and Kellogg (2) from Asia. It differs, *inter alia,* in the posterior portion of the uterus, which in *D. decipiens,* is not pushed backwards into the next proglottid.

D. decipiens has been recorded from Uruguay by Wolffhügel and Vogelsang (16) on the basis of feeding experiments with a plerocercoid which they found in *Didelphis paraguayensis.* A study of the adult cestode convinced them it was *D. decipiens* (Diesing, 1850) (Syn. *D. serratus* Diesing 1850), and they point out that it differs from *D. latus* in its smaller size and absence of a uterine rosette. However, recent work has shown that the rosette shape of the uterus is seen in very few species and that its absence is a sub-generic rather than a specific character (2). Wolffhügel and Vogelsang (16) found the same species in a jaguar *(Felis onca)* and they state that it has also been recorded from Brazil in *Felis yaguarundi* by Parodi and Widakowich under the name of *D. longicollis.*

If Wolffhügel and Vogelsang's species is the same as that described by Faust, Campbell and Kellogg, then the present tapeworm is not the same species as that found in the south of South America.

D. reptans (Diesing, 1850), has also been recorded from South America, occurring as a larva in this host as well as in other carnivores, including *Tayra barbara.* There is some doubt as to the identity of *D. reptans.* Some workers have considered it is identical with *D. ranarum.* If this opinion is correct, the present species definitely differs from it. The whole genus is very imperfectly known and it is regarded as preferable to consider the present form as a new and distinct species, of which the most outstanding character is the shape and disposition of the uterus; accordingly, the name *D. urichi* sp. nov. is proposed for it.

Diphyllobothrium trinitatis sp. nov.

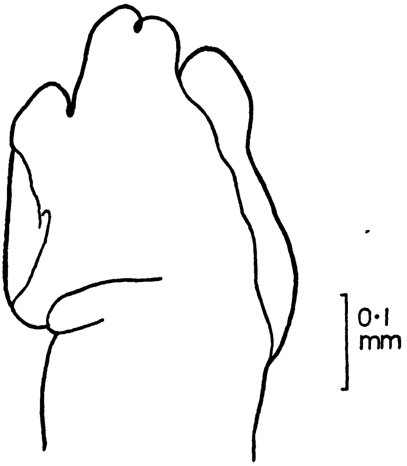
A large number of a second species of this genus of cestodes was recovered from the small intestine of a crab-eating racoon *(Procyon cancrivora).* They were accompanied by a number of obviously recently ingested plerocercoids which are discussed later.

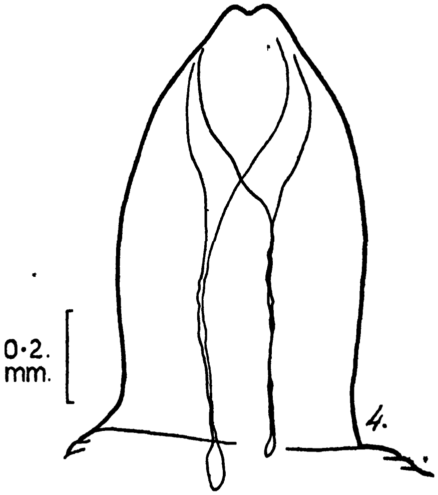
The adult worms are much smaller than the forms found in the ocelot, and none was more than 10 cm. in length.

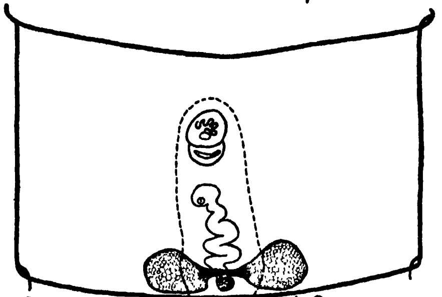
The scolex (Fig. 4) has large bothria on its dorsal and lateral surfaces, and is bluntly conical in shape. There is no neck, segmentation beginning immediately at the end of the bothria.

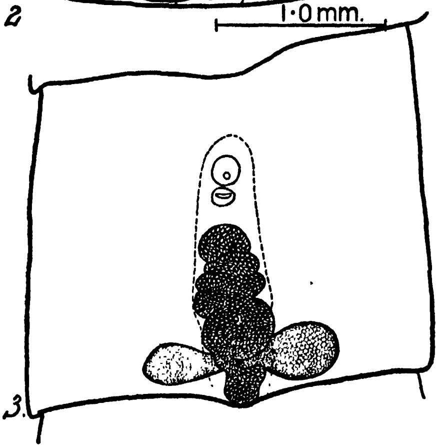
The segments at first are very short and wide but at the posterior end of the chain they are nearly three times as long as broad (Fig. 5).

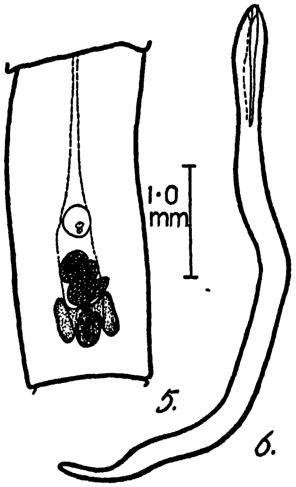
The genital openings are almost in the exact centre of the ventral surface of the segments; the uterine opening is a short distance posterior to this and is almost medial. The uterus is towards the posterior end of the segment and has fewer loops than the previous species: moreover, unlike it, these loops are dorso-ventral rather than lateral. The uterus never extends posterior to the ovary, which is situated a short distance from the posterior margin of the segment.











Diphyllobothrium urichi sp. nov.

FIG. 1. *Scolex.* FIG. 3. *Old mature proglottid.*

FIG. 2. *Young mature proglottid.*

Diphyllobothrium trinitatis sp. nov.

FIG. 4. *Scolex.* FIG. 5. *Mature proglottid.*

Diphyllobothrium sp. inq.

FIG. 6. *Entire parasite.*

N.B.—*In Figures 2, 3 and 5, the testes and yolk glands are* not *shown, the broken line in each case representing their medial limits.*

Both testes and yolk glands are numerous and, as usual, occupy the two lateral fields. Both meet in the mid-line posterior to the ovary. Anterior to the genital openings, however, the testes alone meet in the mid-line, the two groups of yolk glands having a small space between them which extends to the anterior limit of the segment.

The eggs are oval and, while about the same width as those of the previous species, they are a little longer (58μ by $28–30\mu$) giving them a much more elongated appearance.

This species differs markedly from all other described species and it is, in consequence, regarded as new, under the name of *Diphyllobothrium trinitatis.* It may be distinguished by the shape of the head, absence of neck, disposition of uterine tube, and the relative distribution of testes and yolk glands.

Diphyllobothrium sp. inq.

In addition to *D. trinitatis* a considerable number of young forms were recovered from the same host. These (Fig. 6) consist only of a scolex and neck and probably represent recently ingested plerocercoids. The shape of the scolex is of the *D. latum* type and consequently, it has been considered desirable to regard it as a *species inquerenda* rather than refer it to any known form. It may, of course, be the young stage of *D. trinitatis.*

Nematoda

Necator urichi sp. nov.

Four females but no males, of this species were recovered from a racoon *(Procyon cancrivora).* The length varies from 4.5 mm. to 6 mm. The buccal capsule (Figs. 7 and 8) is of the typical *Necator* type. Viewed from the side it is almost triangular in outline, and the mouth opening is directed in a definite dorsal direction. It is guarded by two obvious "cutting-plates." On the ventral wall of the buccal capsule are two small, triangular lancets, and on the dorsal wall, a single, conspicuous dorsal cone.

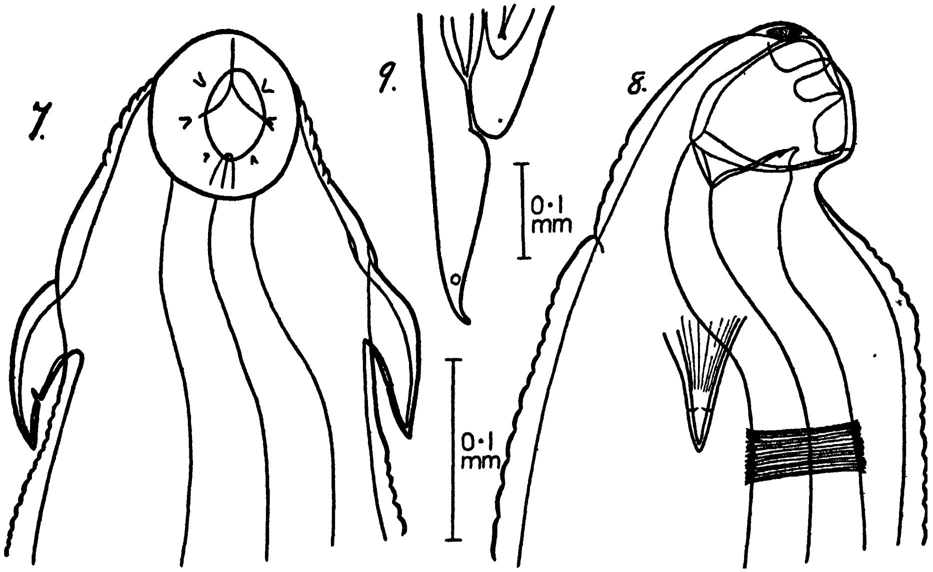
The oesophagus is of the usual type, measuring 0.45 mm. in length and 0.1 mm. at its thickest point.

The excretory pore is situated anteriorly, not far distant from the base of the buccal capsule, while the nerve ring encircles the oesophagus, about 0.15 mm. from its anterior end.

Midway between these two points are two very large cervical papillae—one on each lateral line. They are massive triangular structures which seem like appendages rather than papillae. They are identical on all four specimens so that they cannot be considered as abnormalities. They are sharply pointed, and a short distance from the apex are *two* nerve endings, contained in small depressions, one on the medial aspect of each margin. While the appendages are in all cases held against the sides of the worms, they contain many strands of tissue resembling muscle fibres, suggesting that in life they are movable.

The cephalic and cervical glands are conspicuous and measure about twice the length of the oesophagus.

The ovarian coils are mainly lateral and the vulva is situated just anterior to the middle of the body, which it divides in the ratio of 7 : 8.



Necator urichi sp. nov.
FIG. 7. *Dorsal view of head end.* FIG. 9. *Lateral view of tail of female.*
FIG. 8. *Lateral view of head end.*

The anus is a transverse slit, situated 0.2 mm. from the tip of the sharply pointed tail. Towards the tip of the tail are two small sessile caudal papillae (Fig. 9).

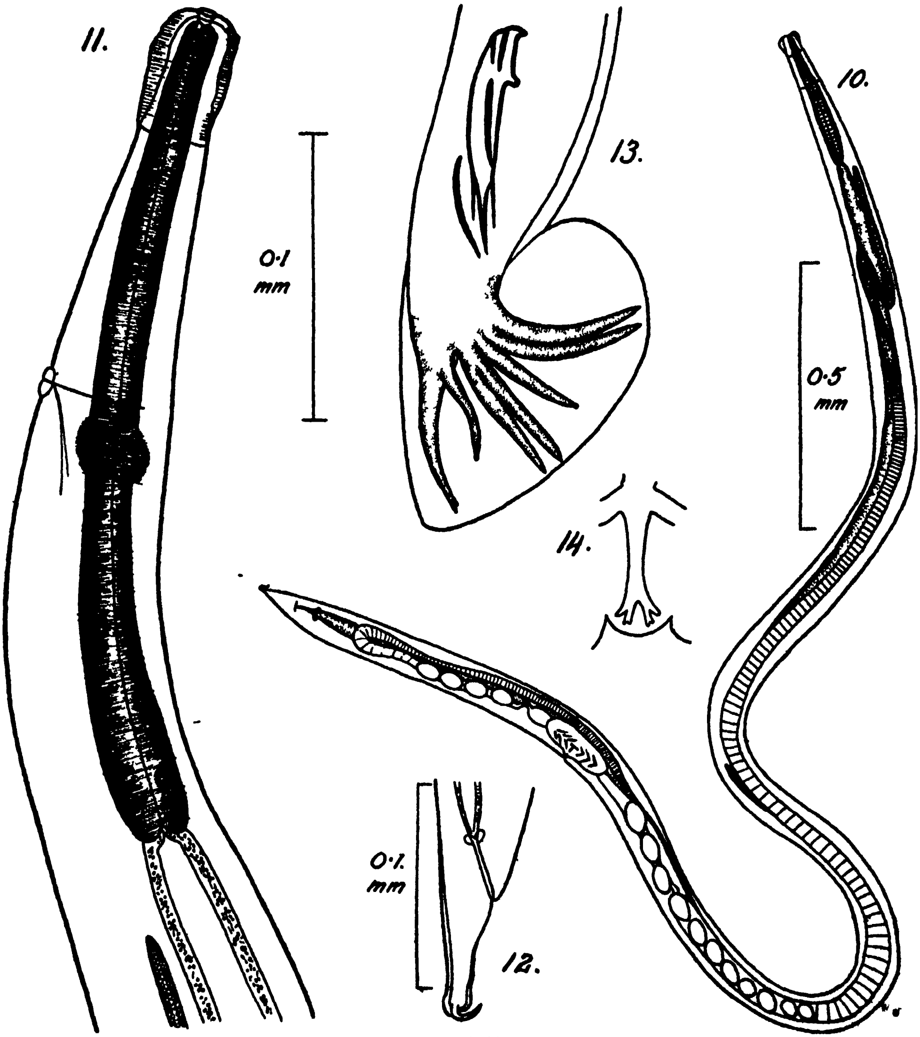
In the absence of males, this species must be referred to the genus *Necator*, although subsequent work may show that it should be removed to a new genus.

Two species of hookworms have been previously recorded from this host in continental South America by Molin (4) in 1860, *viz.*, *Dochmius bidens* and *D. maxillaris*. Professor Leiper has kindly loaned me sketches which he had made from Molin's original material of these two species. These two sketches and Molin's original descriptions show quite clearly that neither is this species. In both, for example, the dorsal cones are absent. The only other hookworm recorded from South American carnivores is *Uncinaria carinii* Travassos (9) in 1915 from *Canis azarae*. Travassos himself draws attention to the fact that this last species is very similar to *U. criniformis* (Goeze, 1782), which also has no dorsal cone. The present species, which is characterized not only by the possession of a dorsal cone, but by the presence of enormously enlarged cervical papillae (amounting to appendages) is accordingly regarded as new and is named *Necator urichi* sp. nov.

Molineus pardalis sp. nov.

Two females and five males of this species were recovered from the small intestine of the ocelot *(Felis pardalis)*.

The females (Fig. 10) have an average length of 3.6 mm. and the males 2.7 mm. The head end is swollen (Fig. 11) and finely transversely striated. There are about 16 main and numerous fine longitudinal striations on the body. The oesophagus is simple and only slightly swollen posteriorly; it is about 0.3 mm. long. The nerve ring surrounds it about its middle point. Cervical papillae are absent. The excretory pore is conspicuous and is situated just anterior to the nerve ring; it lies on a groove (which, however, disappears on the dorsal surface) and is protected by two small lips.



Molineus pardalis sp. nov.

Fig. 10. *Entire female.*
Fig. 11. *Head end, lateral view.*
Fig. 12. *Tail of female.*
Fig. 13. *Bursa of male, lateral view.*
Fig. 14. *Dorsal ray of bursa (to same scale as 13).*

The female genital system is double and simple. The uteri are opposed and terminate in conspicuous ovejectors. Each uterus contains four or five eggs in a single row. The superior ovary is almost straight, originating about the base of the oesophagus. The inferior ovary turns on itself just in front of the rectum; its origin is in the middle of the body. The vulva is 0.65 mm. and the anus, 0.05 mm. from the end of the body, which is slightly swollen (Fig. 12) and then projects as a sharp, simple spine.

The males (Figs. 13 and 14) have a simple bursa, which is continuous ventrally. There are two large lateral lobes and a single small dorsal lobe. The ventral rays are equal and parallel and widely separated from the others. The externo-lateral ray is shorter than the other laterals and does not reach the edge of the bursa. The externo-dorsal rays originate almost at the base of the main dorsal stem and are parallel to the lateral group. The dorsal ray bifurcates near its tip (Fig. 14) to form a long-stemmed Y. Each bifurcation divides into two approximately equal parts, the medial of which are divided terminally. Pre-bursal papillae are absent.

The spicules (Fig. 13) are similar and equal. They are short stout structures, about 0.075 mm. long, terminating in three short sharp spines. The gubernaculum, although about half as long as the spicule, is slender and curved.

This species is smaller than the type species, *M. felineus* Cameron, 1923 (1). It differs from it especially in the relatively large externo-lateral ray and in the shape of the spicules.

Molineus major sp. nov.

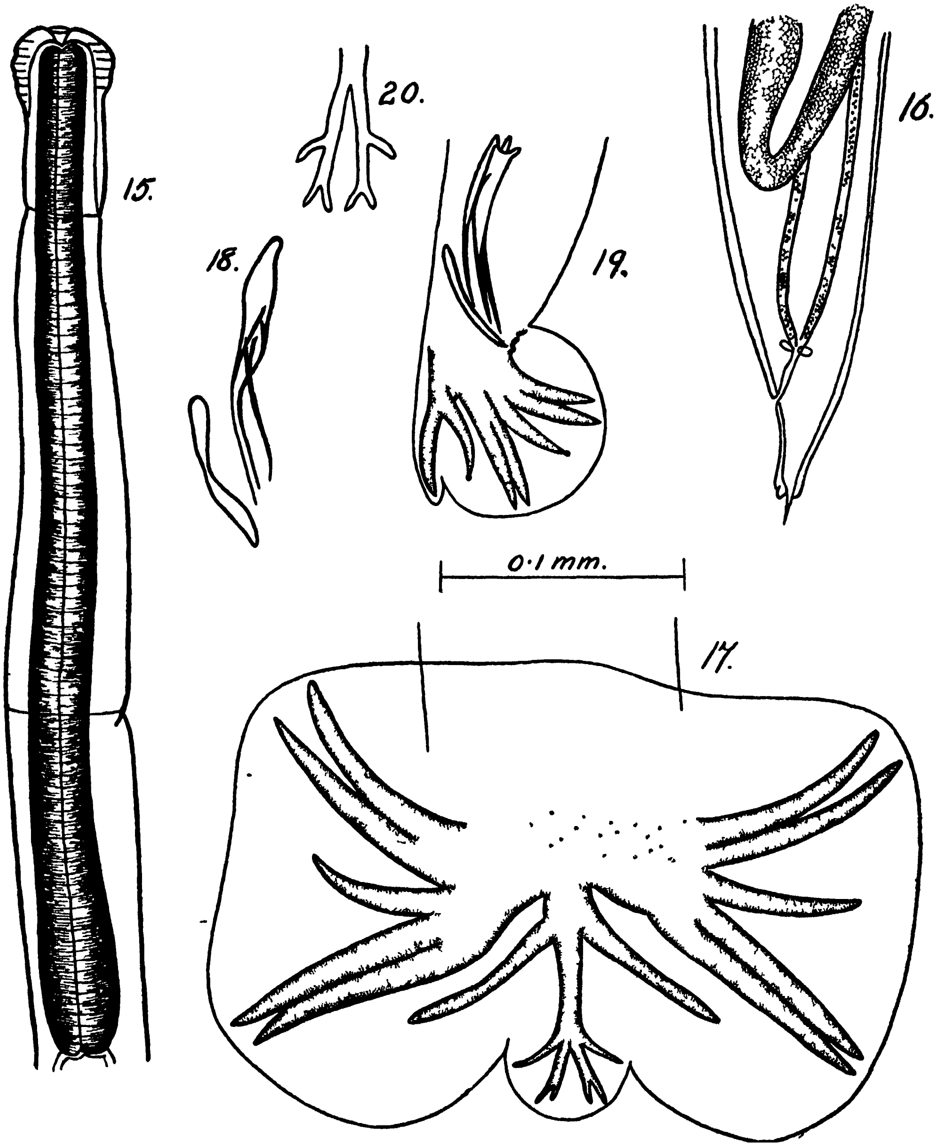
A number of specimens of this species were found in the small intestines of three "wood-dogs" *(Tayra barbara)*. The females measure 8.75 to 9 mm. in length, while the males are little more than half this size, *i.e.*, 5 to 5.5 mm. The maximum thickness of the females is 0.15 mm. and of the males 0.09 mm. The cuticle of the head end (Fig. 15) is swollen, but unlike the previous species, it is conspicuously transversely striated in its anterior portion. The anterior portion moreover is much more swollen than the posterior part. While longitudinal striations are conspicuous in the rest of the body, transverse striations are also present.

Six small circum-oral papillae are present.

The oesophagus is about 0.45 mm. long in the male and 0.55 mm. in the female. The lips of the excretory pore are less conspicuous than in the previous species, but a complete ring is present in the cuticle at this point. In the females this ring is just behind the middle of the oesophagus but in the male it is even farther back, lying at the junction of the middle and posterior thirds.

The vulva in the female is about 1.7 mm. from the posterior end. The vagina is more powerful and the ovejectors longer. The uteri are much more voluminous and each contains a *double* row of eggs—some 50 in each. The anus is about 0.05 mm. from the tail, which is swollen—often with small secondary swellings. The spine is quite conspicuous (Fig. 16).

The bursa in the male is large and rectangular (Fig. 17), and the dorsal lobe is distinct and relatively large. The bursal formula is very similar to that seen in the type species, but the externo-lateral rays are rather longer and more divergent. The dorsal ray splits into four digitations of almost equal length; the inner, however, are more massive and are divided at their tips.



Molineus major sp. nov.

Fig. 15. *Head end.*
Fig. 16. *Tail of female.*
Fig. 17. *Bursa of male, ventral view.*
Fig. 18. *Spicules and accessory piece, side view.*

Molineus barbaris sp. nov.

Fig. 19. *Bursa and spicules of male, side view.* Fig. 20. *Diagram of dorsal ray.*
N.B.—*All drawings, except Fig. 20, are to the same scale.*

The spicules (Fig. 18) which measure 0.13 mm. in length, are very similar to those seen in *M. felineus*. They are very faintly cuticularized. The gubernaculum is about 0.075 mm. long.

This species can be separated from the type species by the points mentioned above, especially by the voluminous nature of the uteri. The name *Molineus major* sp. nov. is proposed for it.

Molineus barbaris sp. nov.

A few examples of a second species of *Molineus* were recovered from two of the wood-dogs. The fact that it was not present in the other, made the separation easy.

It is a much smaller species, the females being only 4.1 mm. and the males 3.4 mm. in length. The head is similar to the last species, but the excretory pore is conspicuous and the ring is anterior to the middle of the oesophagus.

The vulva is situated 0.85 mm. from the tail and the lips are conspicuous. The uteri are much smaller, carrying a single row of 10–12 eggs in each. The tail is similar to the last species, the anus being 0.075 mm. from the tip.

In the male, the dorsal lobe (Fig. 19) is comparatively conspicuous, although the whole bursa is small. The externo-lateral ray is comparatively large, although still smaller than the other laterals. The termination of the dorsal ray (Fig. 20) differs also: there are two relatively long bifurcations, each of which splits terminally; about half way down each bifurcation a curved lateral process is given off.

The spicules, under 0.1 mm. in length, are provided with three long sharp points. The gubernaculum is about half the length of the spicules.

The name *Molineus barbaris* sp. nov. is proposed for this species, which is closely related to the type. It differs from it in several points however—in the complete cervical groove, in the completely separated externo-lateral ray, and in the shape of the dorsal ray. It is easily separated from *M. major* by its much smaller size and from *M. pardalis* by the shape of the spicules and of the dorsal ray.

Molineus sp.

A single somewhat decomposed female of this genus was recovered from the small intestine of a racoon *(Procyon cancrivora)*. It is not possible to refer it to any species, although the shape of the uterus suggests that it may be *M. barbaris*.

The Genus *Molineus* Cameron.

In 1923, the writer described the type species of this genus, *M. felineus* from *Felis yaguarundi* from Brazil. In the original description a cervical groove was stated to be absent, but a subsequent examination of the material, which was not in a good state of preservation, showed that a partial groove was present on the ventral side of the body, and the generic concept must be altered in consequence. *M. felineus* is the only trichostrongyle which has been previously described from South American carnivores and the four species may be distinguished as follows:

	M. felineus	*M. pardalis*	*M. major*	*M. barbaris*
Host	*Felis yaguarundi*	*Felis pardalis*	*Tayra barbara*	*Tayra barbara*
Length—female	5.25 mm.	3.6 mm.	8.75–9 mm.	4.1 mm.
Length—male	4.75 mm.	2.7 mm.	5–5.5 mm.	3.4 mm.
Transverse striations on cephalic swelling	Conspicuous	Fine	Conspicuous anteriorly	Conspicuous anteriorly
Excretory groove	Ventral only	Ventral only	Complete ring	Complete ring
Uterus:				
Eggs	In single row	In single row	In double row	In single row
Bursa:				
Externo-lateral	Half length other lateral rays	Three quarters length other lateral rays	Half length other lateral rays	Half length other lateral rays
	Separate from M.L. for half length only	Completely separate	Completely separate	Completely separate
Terminal digitation of dorsal ray	Conspicuous: lateral largest	Small	Conspicuous	Fairly conspicuous. Lateral small
Dorsal lobe	Indistinct	Indistinct	Distinct	Distinct

Physaloptera maxillaris Molin, 1860

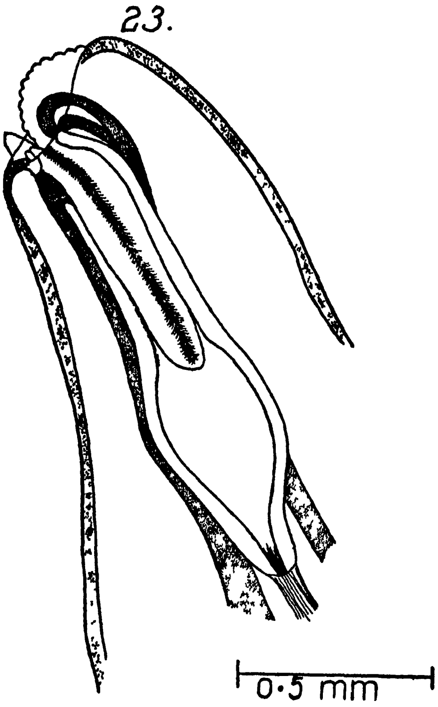
A single female of this species was recovered from the small intestine of *Procyon cancrivora*. It was fully developed but contained no eggs. It agrees in every respect with Ortlepp's (5) excellent description and the writer has nothing to add to it.

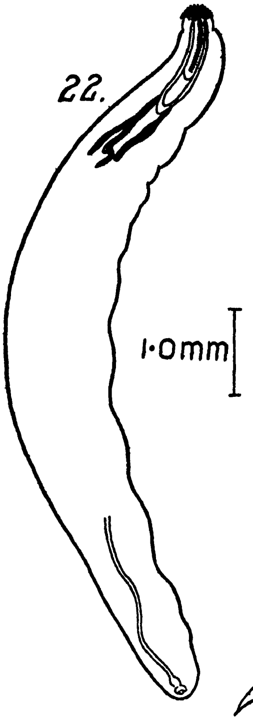
Acanthocephala

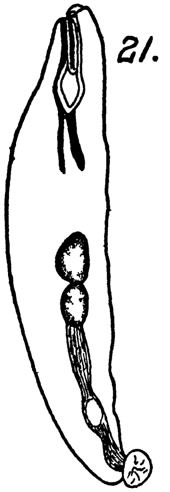
Prosthorhynchus urichi sp. nov.

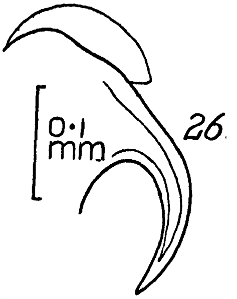
One male and two female acanthocephalid worms were recovered from the small intestine of *Procyon cancrivora*. The male (Fig. 21) is 5.5 mm. long and the females (Fig. 22) about 8.0 mm. The cuticle is thick and smooth and is without spines. The "nuclei" of the sub-cuticula are branched. In all cases the proboscis is retracted, but in one female it is slightly extruded to show a very short neck, which is free from spines. The proboscis is long and cylindrical and is armed with numerous spines, which have long, sharp, recurved points and, so far as can be seen, single roots. The proboscis sheath is nearly cylindrical anteriorly, but is slightly swollen towards its posterior end (Fig. 23). It is inserted at the base of the proboscis, at the anterior end of the body: the proboscis should be completely extrusable. Three lemnisci are present; these are long and slender and about twice the length of the proboscis sheath.

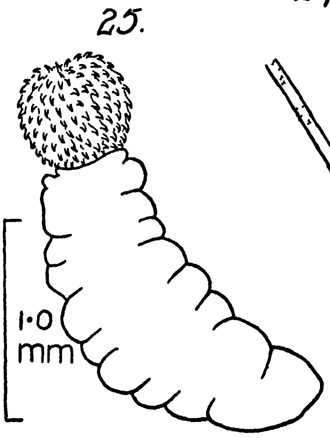
The oval testes in the male lie tandem just behind the mid-point of the body, with their long axis in the direction of the body. The writer was unable to be certain of the details of the cement gland (prostate gland) in

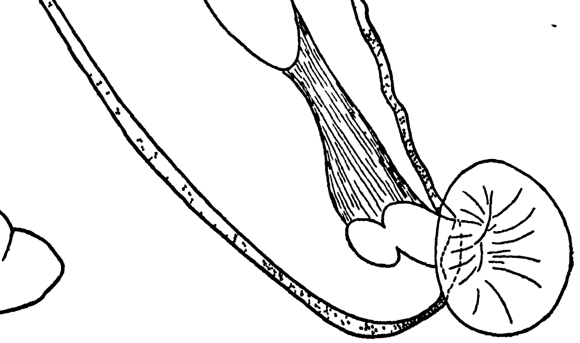












Prosthorhynchus urichi sp. nov.

Fig. 21. *Entire male.*
Fig. 22. *Entire female.*
Fig. 23. *Head of male.*
Fig. 24. *Tail of male.*

Paridosentis sp.
Fig. 25. *Entire animal.*

Porrocephalus stilesi Sambon.
Fig. 26. *Outer hook.*

the single male, although it seems to form a single, elongated mass. A simple campanulate bursa is present (Fig. 24).

The two females are both immature and the egg masses were only in process of breaking down into eggs. One ovary lay just behind the proboscis sheath: a second could not be seen but a number of egg masses were scattered throughout the body cavity. The uterus is long and narrow, but the uterine bell could not be observed. The vaginal sphincter is small as is the vagina proper. The genital opening is sub-terminal.

These worms seem closely related to the genera *Plagiorhynchus* and *Prosthorhynchus*, none of the described species of which, however, possess a neck (7, 10, 11). Although unable to be certain of the structure of the cement glands (which is essential under our present criteria for the determination of genera of Acanthocephala), the writer is tentatively referring it to the genus *Prosthorhynchus*, pending the discovery of more mature specimens. As all the described species have so far been found in birds, it is possible that this also is a bird form in an abnormal host. Although some of the structures necessary for a correct conception of a species have not yet been seen, it is proposed to regard this form as a new species, *Prosthorhynchus urichi* sp. nov., with the following tentative diagnosis:— Body elongated, without spines, with short unarmed neck and long cylindrical proboscis which can be completely inverted and which carries numerous spines: three lemnisci: testes in middle region of body: cement gland elongated, but number of elements undescribed.

Paridosentis sp.

In addition to the forms described above, another acanthocephalid was recovered from the same host. This (Fig. 25) is obviously an immature form. It, however, closely resembles Van Cleave's description (13) of *Paridosentis iracundus* Van Cleave, 1920, from Venezuelan fish and it is provisonally referred to this genus, although absence of more mature forms prevents a specific determination.

Arthropoda

Porrocephalus stilesi Sambon, 1910

A single linguatulid was found in the small intestine of the ocelot *(Felis pardalis)*. It corresponds very closely to Sambon's description (6) of *Porrocephalus stilesi*, which occurs in the Bushmaster and Fer de Lance (both of these snakes are present in Trinidad, although I am unaware of any parasites having been recorded from them there). It is a young female, only 10 mm. long. There are about 50 annuli, and the tail, including the anal and female openings, closely resembles Sambon's drawing. The mouth is oval, but somewhat constricted anteriorly. The hooks are large, the inner pair being slightly larger than the outer. The straight accessory spine, described by Sambon as being present on the outer hooks, was not present: there was, however, a small piece of tissue present on each outer hook in the place where the spine should be (Fig. 26). As the specimen is obviously a very young one, it is possible that this tissue represents the spine in the fully grown adult, which reaches a length of 80 to 96 mm.

The nymphal stages occur in mammals in other members of this genus, although they have not yet been described for this species and it is probable that this parasite is an accidental form in the ocelot. The same animal had, in its intestine, several decomposed trichostrongyles which are normally found in agouti, as well as remains of agouti in its stomach. The pentastome possibly came from the same source. Both snakes mentioned above are also known to feed on agouti.

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SPERMATOCYTE CHIASMA FREQUENCY IN STRAINS OF MICE DIFFERING IN SUSCEPTIBILITY OR RESISTANCE TO THE SPONTANEOUS OCCURRENCE OF MALIGNANT TUMORS[1]

By C. Leonard Huskins[2] and E. Marie Hearne[3]

Abstract

Significant differences have been found in the spermatocyte chiasma frequencies of ten strains of mice differing in genetic susceptibility or resistance to the spontaneous occurrence of malignant tumors. In three resistant and five susceptible strains there is a positive correlation between susceptibility and low chiasma frequency, and between resistance and high frequency. Two interrelated strains may be exceptional, but their cancer and leukemia records are not clearly established, and one of them, though classed as resistant, is somewhat anomalous genetically.

The study comprises a total of 16 tests, each made on 25 complete cells. It was made as an indirect test of a working hypothesis on the mechanism of genetic susceptibility. This hypothesis is based on: (a) the assumed validity of the somatic cell mutation theory of cancer in its most general formulation, (b) the conclusion that gross chromosome irregularities commonly found in tumor cells cannot in themselves be the cause of cancer, though they may be a direct result of some less obvious lack of correlation in the mitotic process, (c) the fact that in different organisms the existence of genes controlling chromosome structure and behavior and other aspects of cell division has been established, (d) the observation that in tumors themselves the chromosomes are commonly found to be "split" precociously, and (e) the observation that "splitting" of meiotic chromosomes is correlated with the absence of chiasma formation. The hypothesis postulated that in strains of mice genetically susceptible to the spontaneous occurrence of tumors, though not yet having cancer, the chromosomes might be found to be "split" slightly in advance of the normal period, and that this could be measured indirectly, but most precisely, by its anticipated effect of causing lowered chiasma frequency at meiosis. The precocious "splitting" would itself be a primary result of some genic difference in the constitution of susceptible animals and might either—through causing grosser chromosome irregularities—lead directly to neoplastic growth, or be a primary morphological concomitant of an underlying cause common to both phenomena.

Introduction

The idea that chromosome aberrations, which are so frequently observed in tumors, are themselves the cause of tumor development has been very widely held since it was first mooted in 1891 by von Hansemann (25) and shortly afterwards developed as a general theory by Boveri (10, 11). Winge (55, 56) and others have stressed the causal significance of polyploidy—the multiplication of whole chromosome sets—rather than aberrations involving irregular chromosome numbers. On the other hand, it has been as widely argued that both of these types of nuclear change are the result of the conditions under which tumors develop, and not the cause of their development. Andres (2) may be cited as one of a number of workers who have shown clearly that none of the major aberrations of mitosis are specific for cancer and that in both primary tumors and metastases there may be normal chromosome number and form.

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Chimaeras of plants or animals provide abundant evidence that most cytological aberrations do not cause cancer. In chimaeras generally there is a tendency for the patches of tissue made up of aberrant cells to be crowded out by normal cells, and Gowen (23) has pointed out that the aberrant tissues in *Drosophila* gynandromorphs do not show uncontrolled growth.

Seecof (52) showed that in a new transplantable tumor of the rat, giant (polyploid) cells first appeared in the eighth transplant generation; that the largest number of giant cells occurred in tumors that grew rapidly at first and then stopped; that transplantations rarely "take" after this, and that if they do "take" they show no giant cells in the transplants. This, with other studies, seems to make a fairly conclusive case against the polyploidy hypothesis of cancer causation. In crown gall of plants, polyploid cells may be of significance, but, as Levine (34) points out, this is a "protective and reparative process" whereas, he concludes, "Cancer is a disease of the cell which manifests itself in division."

There is, however, one cytological peculiarity of tumor cells which might, *a priori*, be expected to have some more general relationship to the cause of cancer. This is the precocious splitting of some chromosomes, or, more properly (since it is now clear that chromosomes may split one or more division cycles in advance of that in which their separation occurs, (*cf.* 28)), the precocious separation of daughter half-chromosomes before metaphase which was first emphasized by Ludford (39, 40). He observed it in the Jensen Rat Sarcoma, Mouse Sarcoma 375, Fibiger's Tar Sarcoma and Mammary Carcinoma 63. It was the observation by the senior author in 1929 of this precocious "split" in Dr. Ludford's preparations that led eventually to the formulation of the working hypothesis to test which the studies here reported were undertaken. Similar precocious "splitting" of the chromosomes, besides tetraploidy and other aberrations, was also seen in preparations of the Walker Rat Sarcoma kindly demonstrated to the senior author by Dr. Margaret Lewis in 1931. Precocious "splitting" was found by Lewis (35) in tissue cultures of several spontaneous tumors of the mouse also but at that time she was inclined to consider malignancy as bound up with some permanent alteration of the centrosome apparatus, and the chromosome abnormalities as secondary (36). Lately, Lewis and Strong (37) have demonstrated precocious chromosome "splitting" in tissue cultures of 51 spontaneous tumors, all carcinomas of the breast, from seven strains of mice. Of these they state, p. 86, "The growths from the 51 tumours taken from seven strains of mice exhibited chromosomes that were so widely split during prophase and metaphase that they often appeared as pairs of chromosomes of the same shape and size. The longitudinal division of the chromosomes was sometimes evident in the early prophase, usually in the late prophase, and practically always in the metaphase. At the beginning of anaphase the halves of the chromosomes became separated, one half going to each anaphase plate. The split condition of the chromosomes was evident in mitotic figures in which the number of chromosomes was increased, as well as in those that had the normal number (Figs. 27 and 34). The chromosomes that lagged behind during the migration to form the metaphase were split (Fig. 36), but those that lagged in the separation to form the anaphase, telophase, and daughter nuclei were usually separated and appeared singly rather than split (Fig. 39)."

They comment later on the uniformity of the general cell type in these spontaneous mouse tumors and emphasize that the results are different from what they have found in tissue cultures of human tumors and of the spontaneous tumors of Dr. Walker's inbred rats. In the discussion section of this paper written by Dr. Strong he states: "Thus from the study of tissue cultures it is evident that peculiarities of chromosome behaviour may be not fundamental but only coincident or the result of the original production of certain types of cancerous tissue. Thus the intrinsic factors, such as must be assumed to explain the findings of transplantation, may also be called upon to explain the retention of tissue specificity found to persist in tissue cultures."

Potter and Richter (50) found precocious separation of chromosome halves to be very marked in the mitotic divisions of lymphocytes of mice affected with lymphatic leukemia.

Though precocious chromosome "splitting" may be a general characteristic of malignant cells, so long as it is observed only in the malignant cells themselves there remains no way of deciding whether it is causal or resultant.

On the other hand, should any degree of precocious separation be found in normal cells of animals highly susceptible to the occurrence of spontaneous tumors, and not in resistant strains, it would clearly indicate that precocious separation is either directly causal or primarily associated with the cause of malignancy. The fact that genetically pure strains of mice differing greatly in their degree of susceptibility to spontaneous tumor formation now exist, and the readiness of Dr. C. C. Little of the Roscoe B. Jackson Memorial Laboratory to supply other workers with many of these strains, made it possible for us to examine this aspect of the cancer problem.

Before proceeding to a report of the data obtained, it may, however, be well to summarize briefly other evidence which indicates either directly or by analogy that malignancy arises through a change in the nature of individual cells, and that such changes are of a genetic nature, since these are the primary postulates underlying our study.

The somatic cell mutation theory of cancer (using the term "mutation" in its widest sense as implying some undefined type of permanent change in the hereditary material) appears to have been gaining ground steadily from the time of Ross (51), Oertel (48), Bauer (4) on to the current work of Curtis, Dunning and Bullock (15–17), which appears particularly conclusive. Very complete reviews of the evidence available up to their time of publication have been presented by Ludford (40) and Levine (34). Some direct, though not conclusive, evidence is provided by Mottram's (43) experiments in inoculating mice with X-rayed testicular tissue, in the course of which one tumor, apparently caused by an X-ray mutation, was produced. In their work on rats with *Cysticercus fasciolaris*, the larval form of *Taenia taeniaeformis* which is the common tape worm of the cat, Curtis, Dunning, and Bullock (16) have shown that though the host may have from one to one hundred benign cysts, malignancy is usually confined to a single cyst. "Of 4,321 cysticercus tumours, 79.3% were sarcomata of the large-cell type and probably arose from the cells which formed the inner zone of the cyst wall, 20.6% were sarcomata composed of smaller cells and possibly arose from the outer zone of the cyst wall, 6 were adenomata, and one was a carcinosarcoma which must have arisen from the snared off bile duct and liver epithelium which was embedded in the wall of the cyst." They conclude that "If the change of a normal to a tumour cell results from a process analogous to somatic mutation which is brought about by the chance action of an irritant it would be expected that the types of cysticercus tumours observed would represent the various types of cells found in the cyst wall and that each would be represented by a number proportional to the comparative number of these cells exposed to the irritant and their relative proximity to it. The above classification [see their Table IX] shows that they roughly approximated this expectation."

Evidence from analogy for the genetic nature of malignancy is provided by the occurrence of a gene in *Drosophila melanogaster* which produces tumors; the occurrence of melanotic neoplasms in intergeneric hybrids of *Platypoecilus* and *Xiphophorus* (21); of tumors on interspecific hybrids of birds (Poll, 1920, cited by Kostoff (32)); of *Xeroderma pigmentosa* produced by a recessive gene in human beings; and of tumors resembling crown gall tumors on interspecific hybrids of *Nicotiana glauca* and *N. langsdorfii* (32, 54). Jones (31) has pointed out some possible analogies with chromosome deficiencies in maize and other organisms. Mottram (44) has obtained irregularities of mitosis as a result of treatments with tar and gentian violet.

The now fairly long list of cases known in which genes affect either the mitotic or meiotic processes and the mechanism of cell division provides further evidence *by analogy* that malignancy may be due to mutation. Amongst these may be cited the recessive gene causing long meiotic chromosomes in *Matthiola* (33) which has other correlated effects also (3, 49). In *Drosophila melanogaster* there are genes affecting crossing over and somatic elimination of chromosomes (12, 22, 53, and others). In maize, Beadle (5–7) has found different genes causing asynapsis, failure of cytokinesis, and polymitosis. Asynapsis caused either by genes or chromosome deficiencies is known also in at least a dozen other plants (see 26 for some of these). In *Allium*, interstitial localization of chiasmata in chromosome pairing is genetically determined, probably by a single recessive factor (20). It is thus clearly demonstrated that the chromosomes carry genes necessary for self-regulation during karyokinesis, and there is therefore, by analogy, a strong *a priori* case for the assumption that loss of regulatory genes may cause malignancy.

Materials, Methods, and Observations

Observations were originally made upon spermatogonial cells of male mice four to five weeks old, for the reason that these are among the largest and easiest of mammalian somatic cells in which to study mitosis and chromosome form and behavior. It has since appeared that for our purpose of studying the degree of chromosome separation before metaphase, cells in which the chromosomes are less contracted may be preferable. With the fixing and staining technique here described, and various modifications of it, lymph-gland leucocytes have been found to provide better preparations for the detailed study of mouse chromosome shape than any other tissue or method yet tried; other difficulties, however, arise. In the spermatogonial cells here reported on, separation of chromosome halves appeared often to be slightly precocious in strains of mice highly susceptible to the spontaneous occurrence of tumors, but the difference, if any, between susceptible and resistant strains was too slight and too variable to afford any conclusive evidence.

An indirect test of possible differences in the time of somatic chromosome splitting or separation was therefore made, on the hypothesis that precocity of splitting is correlated with a reduction in the tightness of chromosome pairing during meiosis or, in current cytological terminology, with a reduction in frequency of chiasma formation during the meiotic prophase. Data obtained from asynaptic wheat and oats (26) and from *Fritillaria meleagris* (29) provided the basis for this subsidiary hypothesis. Whatever the merits of this hypothesis, the data obtained in applying it to the strains of mice studied appear to have significance for the cancer problem.

The description of the strains used, as given in the printed leaflet provided by the Roscoe B. Jackson Memorial Laboratory, is as follows.

Strain A. Albino mice in which there is a high incidence of spontaneous mammary tumors among breeding females at an average age of nine to twelve months. Inbred since 1918.

Strain D. Dilute brown mice differing from wild mice by three mendelian characteristics, a strain high in the incidence of spontaneous mammary tumors at an average age in breeding females of 10½ months. Inbred since 1909.

Strain C 57 Black. Differing from wild mice by one mendelian recessive characteristic. A high fertility strain in which, during the last ten years there has been no record incidence of carcinoma of the breast. Inbred since 1921.

Strain M Leaden. Mice differing from wild stock by three mendelian recessive characteristics. A high fertility strain with extraordinarily low cancer incidence. Inbred since 1921.

Strain Y. Yellow or brown mice with good fertility, a physiological tendency to a high degree of adiposity and a relatively high occurrence of spontaneous sarcoma of different types. Inbred since 1925.

Strain C-3-H. Colored like the wild house mice. High fertility and high incidence of spontaneous mammary tumors. Average age of tumor incidence eleven months. Inbred since 1921.

Strain CBA. Wild type color. High fertility. Have been selected toward longevity for fourteen generations. No tumors of any descriptoin in individuals whose average age of life for the past ten generations is twenty-one months. Inbred since 1921.

Strain N. Four recessive factors, dilute, brown, non-agouti, piebald. No tumors observed for ten generations. Average age at death is eighteen months. Inbred since 1921.

Strain I. Carry five recessive factors, the same as in strain "N" with pink-eye additional. No tumors have been observed in twelve generations. Inbred since 1921.

Strain C 57 Brown. Have tumors at an advanced age, medium fertility. Inbred since 1921.

The "susceptible" strain designated "Carrel's C 57 Black" in the present study was likewise obtained from Dr. Little who wrote (January 11, 1935) concerning it: "I am accepting the statement of its susceptibility from Alexis Carrel of the Rockefeller Institute. None of the mice of this stock in this laboratory have reached an age when first-hand information is available."

Various fixatives were used in preliminary studies. The most satisfactory method of preparation, and the one used exclusively after the first five strains were examined, was to kill the animals by decapitation, quickly remove one of the testes, cut it into three pieces, smear each *immediately* (speed is essential) on a clean glass slide and plunge this face downwards (in a dish with two ribs on the bottom to prevent the smear touching it) into Kaiser's solution: 10 gm. bichloride of mercury; 3 cc. glacial acetic acid; 300 cc. distilled water. This was then repeated as quickly as possible with the other testis. After about two hours in Kaiser's solution the slides were transferred to Navashin's fixative and kept in it overnight (8, p. 244). They were then stained by a modified iodine crystal-violet method: after washing in tap water, average pH 7.5, the slides were stained for 15 minutes in 1% aqueous crystal violet. They were then rinsed in tap water and plunged into 95% ethyl alcohol for about 5 seconds. From this they were transferred to an 80% alcohol, 1% iodine, and 1% potassium iodide solution for about 45 seconds, and then passed slowly through 95% and absolute alcohol into a mixture of absolute alcohol and clove oil, equal parts. They were then differentiated under the microscope and washed for at least one hour in four changes of xylol and mounted in Canada balsam.

Observations were begun on Strain A (susceptible) from which chiasma frequency counts were made on 15 primary spermatocyte cells. Fifteen were then examined from strain CBA (resistant), then 10 from A, followed by a further 10 from CBA (see 27). In these first two strains the counts were made first independently by both authors and then jointly, and in cases where any doubt existed the most probable number was decided on. For later strains the maximum and minimum (within the standard used) were recorded for doubtful bivalents. Calculations of significance made by using alternately the higher and lower values for successive nuclei were not appreciably different from those presented, in which minimum values have been used throughout. Apart from the maxima and minima of doubtful cases the standard used throughout was that of "minimum chiasma frequency". That is to say, each bivalent configuration was assessed as having the minimum number necessary for the formation of such a configuration. Certain bivalents have quite certainly had more chiasmata than are assessed, but the arbitrary minimum standard is the only one that can be maintained successfully. Since different workers may have different standards, or may prefer to count the chiasmata at different stages of meiosis, it is only the relative value for different strains evaluated by the same standards which are significant, and not the absolute values per nucleus. This has already become obvious from disagreements between different cytologists working on maize. It is also to be expected, and there is some supporting evidence, that chiasma frequency will differ with the age of the animals (see also Crew and Koller (14)) and with temperature or other environmental influences, as does its genetic correlative, or partial correlative, the crossing-over frequency. The frequencies reported probably do not, however, represent the initial formation frequency and adequate chiasma counts cannot, by present methods, be made at the earliest diplotene stages in mice. For the Wistar Rat, Bryden (13) gives terminalization coefficients of 0.475, 0.503, and 0.577 for ages 2, 8, and 17 months, which he considers "point to a difference in the original frequency of chiasma formation" at these ages.

It should be added further that the term chiasma is here used in the sense understood by Darlington (18) and his school in which all points of intimate contact between paired homologous chromosomes in meiosis are judged to be either "interstitial chiasmata" or "terminalized chiasmata" according to their position. Actually, following studies of *Trillium* (30) the senior author has come to agree with Belling, Metz, and others that terminal junctions are *not necessarily* chiasmata or the result of chiasmata. In a strict sense we now describe as "chiasmata" only those junctions, either terminal or interstitial, between homologous chromosomes at which all four chromatids of the bivalent can be seen and at which two of the chromatids exchange partners. However, pending the acquisition of further knowledge regarding limitations of the chiasma theory of pairing, it is simplest to call all points of contact chiasmata, even though our present viewpoint prevents us attributing significance to terminalization coefficients etc. that might be derived from these data. In the present study only those cells in which the chiasma frequency of every bivalent can be determined have been considered. Since previous studies of chiasma frequency in the mouse have been made on incomplete cells in sectioned material the results are not significantly comparable. Where chiasmata were counted at widely different stages of diakinesis in the earlier part of this study, it was of course necessary, on account of reduction through terminalization, to assume that equivalent numbers of cells at the various stages were included in the totals compared. The later counts were all made at mid to late diakinesis.

In Table I, the data on number of animals, their ages, and the fixatives used are presented.

During the course of this study a strong general impression has been gained that both in mitosis and meiosis the high-chiasma-frequency strains have their chromosomes less contracted than those of low-frequency strains at comparable stages. It has not, however, been possible to obtain significant comparable measurements.

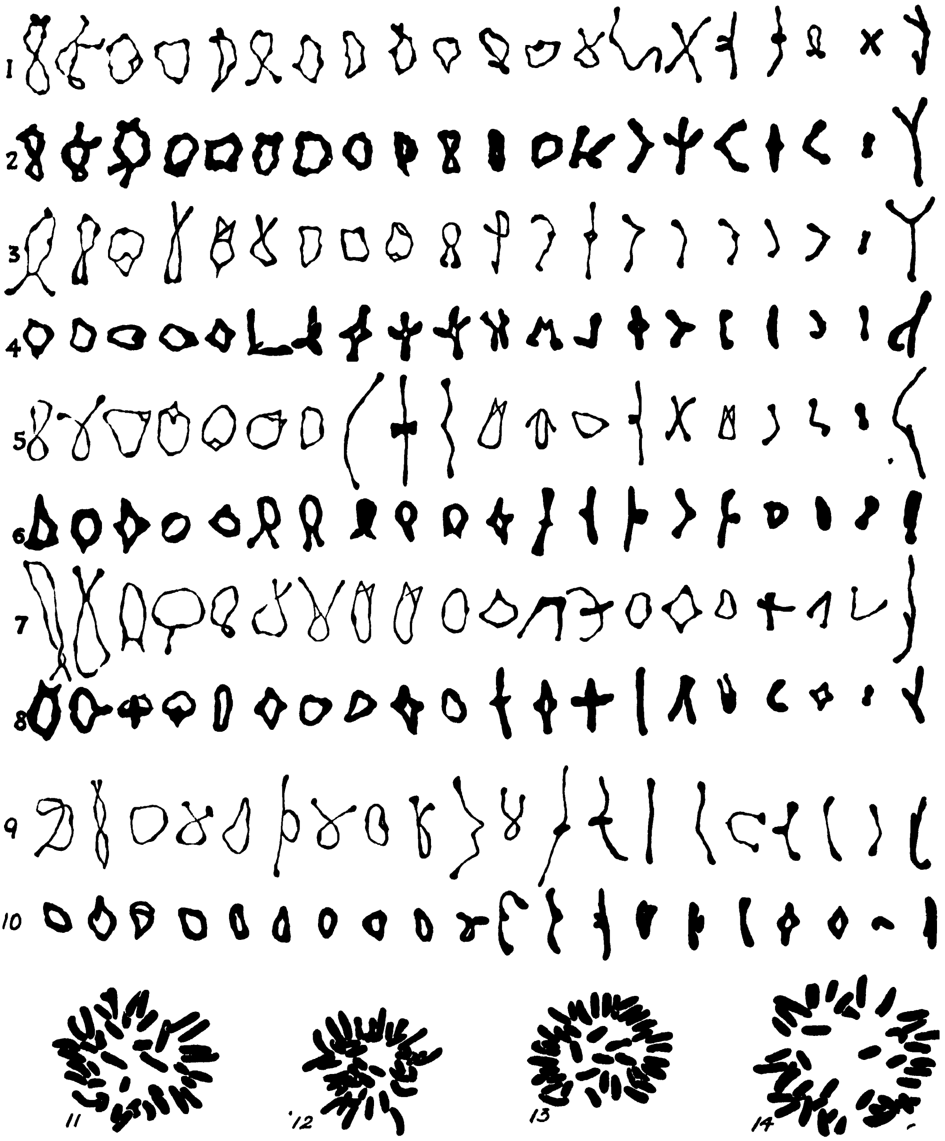

FIG. 1. *Primary spermatocyte chromosomes at early and late diakinesis stages. Each row illustrates the complement of a single nucleus; the total "chiasma frequency" is given in brackets as follows: 1(36), 2(35), 3(31), 4(25), 5(32), 6(30), 7(34), 8(20), 9(32), 10(30). All drawings made at 5800—, reduced to 3700× in reproduction.*

1 : Strain CBA, early diakinesis 2 : Strain CBA, late diakinesis; 3 : Strain A, early diakinesis; 4 : Strain A, late diakinesis; 5 : Strain Y, yellow, early diakinesis; 6 : Strain Y, yellow, late diakinesis; 7 : Strain Y, black, early diakinesis; 8 : Strain Y, black, late diakinesis; 9 : Strain C57 Black, early diakinesis; 10 : Strain C57. Black, late diakinesis; 11–14 : Spermatogonial mitotic metaphases of Strains, A, CBA, C57 Black, and Y, black, respectively.

TABLE I

RECORD OF ANIMALS USED FOR CHIASMA FREQUENCY TEST

Strain	No. of Mice	Approx. ages in months	Fixative
A, First Test	4	1, 1, 4, 4	(a) 2 BE 2 hours; Navashin overnight (b) 2 BD 2 hours; Navashin overnight
CBA, First Test	6	1, 1, 2, 2, 2½, 4	(a) Navashin overnight (b) 2 BE 2 hours; Navashin overnight
Y Black	2	2, 2	(a) 2 BE overnight (b) 2 BE 2 hours; Navashin overnight
Y Yellow	1	2½	(a) 2 BE overnight (b) 2 BE 2 hours; Navashin overnight
C 57 Black, 1st Test	2	1, 1	(a) 2 BE overnight (b) 2 BE 2 hours; Navashin overnight
I	3	2, 2, 2	Kaiser's 2 hours; Navashin overnight
N	4	2, 2, 2, 2	Kaiser's 2 hours; Navashin overnight
A, Second Test	1	4	Kaiser's 2 hours; Navashin overnight
D	2	1, 1	Kaiser's 2 hours; Navashin overnight
M Leaden, 1st Test	1	1	Kaiser's 2 hours; Navashin overnight
C-3-H	2	1, 1	Kaiser's 2 hours; Navashin overnight
C 57 Brown	2	1, 1	Kaiser's 2 hours; Navashin overnight
Carrel's C 57 Black	2	1, 1	Kaiser's 2 hours; Navashin overnight
C 57 Black, 2nd Test	1	8	Kaiser's 2 hours; Navashin overnight
CBA, 2nd Test	1	8	Kaiser's 2 hours; Navashin overnight
M Leaden, 2nd Test	1	2	Kaiser's 2 hours; Navashin overnight

In Table II the data obtained from 16 distinct studies of "chiasma frequency", each made on 25 cells in 11 different strains of mice, are presented. It will be noted that two studies each have been made on CBA, C 57 Black, A, Y, and M Leaden. As mentioned, A and CBA were the first two strains examined. The second set of observations on these strains was made about a year after the first set in order to check any possible change produced by changes in technique or increased facility in observation or, in the case of CBA, by age. Strains C 57 Black and M Leaden were double-checked because they are reported to be resistant and their chiasma frequency was found to be low like that of the susceptible strains. Strain Y is heterozygous for A^y since this gene is lethal when homozygous. Little (38) has shown a significantly higher incidence of mammary tumors in its non-yellow segregates, but not of other types of tumors. The yellow segregates have a chiasma frequency 0.84 higher than that of the blacks; this, though below the standard of significance, we have selected, is in accord with the difference in tumor incidence.

TABLE II

	N	I	CBA, first test	CBA, second test	C 57 Black, first test	C 57 Black, second test	Y Yellow	Y Black	Carrel's C 57 Black	C-3-H	D	C 57 Brown	A, first test	A, second test	M Leaden, first test	M Leaden, second test
	34	33	36	32	31	34	31	33	26	28	26	26	27	26	29	26
	33	35	32	29	33	29	31	28	28	29	24	30	31	30	28	28
	36	33	31	33	30	28	29	30	25	28	25	26	32	27	23	27
	36	35	33	33	33	28	29	31	25	30	25	29	25	31	29	29
	37	34	34	31	28	27	27	31	28	29	25	29	33	30	31	30
	38	36	34	34	33	34	31	33	31	32	31	26	29	32	26	27
	38	37	32	32	28	28	29	31	25	30	29	28	32	29	28	29
	34	34	35	32	29	30	30	28	29	30	30	30	29	28	32	27
	34	34	34	32	29	28	33	31	26	30	29	26	31	31	28	28
	33	35	33	32	31	31	25	26	25	31	28	28	29	29	27	30
	33	34	36	32	28	31	28	28	26	28	26	28	30	30	27	25
	36	31	33	35	30	29	29	29	26	27	28	26	29	27	24	26
	33	32	33	29	29	29	32	32	24	27	28	26	25	30	32	25
	36	32	31	30	29	30	32	30	26	27	26	29	25	30	29	28
	32	36	32	27	28	29	31	26	27	30	27	28	31	28	25	24
	38	32	32	30	31	33	32	26	25	29	28	28	32	28	29	24
	37	33	33	30	32	31	29	29	28	28	28	25	28	29	29	25
	33	31	35	34	29	32	32	27	27	32	27	27	29	31	30	24
	34	35	30	32	30	28	30	29	26	26	29	29	27	30	28	25
	35	33	29	31	31	26	31	30	26	29	28	29	28	30	27	30
	34	36	31	35	33	29	29	27	26	32	31	29	26	31	25	26
	38	33	31	34	31	28	29	32	28	33	25	27	28	29	28	28
	31	35	30	35	31	30	29	28	29	30	27	28	25	28	26	27
	34	34	34	30	29	31	31	27	28	28	26	32	26	29	27	25
	36	35	31	29	32	30	31	27	28	26	29	28	24	28	27	24
Sum	873	848	815	793	758	743	750	729	668	729	685	697	711	731	694	667
Mean	34.92	33.92	32.60	31.72	30.32	29.72	30 00	29.16	26.72	29 16	27.40	27.88	28.44	29.24	27.76	26.68
Sum χ^2	30585	28826	26653	25263	23052	22183	22578	21373	17914	21345	18857	19497	20387	21427	19386	17891

Grand Total = 11891   *Total sum of (variates)² of all columns = 357217*   *Necessary difference for P = 0.01 = 1.40.*

The data have been analyzed by Fisher's method for the Analysis of Variance, and P = 0.01, which requires a difference of 1.4, has been selected for the estimate of significance; in view of the difficulty of obtaining absolutely accurate counts, even the significance of 99 : 1 odds cannot, we consider, be taken too literally. The differences are set forth in Table III.

Discussion

It is apparent from Table III that all the strains listed by the Roscoe B. Jackson Laboratory as having a high incidence of spontaneous tumors have also a low chiasma frequency. Strains A, D, and C-3-H, have "a high incidence of spontaneous mammary tumors", Strain Y has "a relatively high occurrence of spontaneous sarcomata of different types", and Strain C 57 Brown "has tumors at an advanced age." Their chiasma frequencies are: 28.4 and 29.2 in the two tests of Strain A, 27.4 in D, 29.2 in C-3-H, 30.0 in Y yellow, 29.2 in Y black and 27.9 in C 57 Brown. Carrel's C 57 Black which is also, unofficially, reported as susceptible has likewise a very low chiasma frequency—26.7.

The highest chiasma frequencies, 34.9, 33.9, and 32.6, occur in strains N, I, and CBA, which are respectively described as having "no tumors observed for 10 generations"; "no tumors . . . . . . observed in 12 generations"; and "no tumors of any description in individuals whose average length of life for the past 10 generations is 21 months." This latter strain has been "selected toward longevity for 14 generations." In these nine strains there is, then, a correlation between susceptibility to spontaneous tumor formation and low chiasma frequency, on the one hand, and, on the other, between high chiasma frequency and immunity (so far as records are available) from spontaneous tumor formation.

In two other strains reported as resistant, the chiasma frequency is, however, low and the correlation does not hold. At the same time it appears that the genetic and tumor-incidence characteristics of these strains may possibly also be partly anomalous. With the kind permission of Dr. C. C. Little and others concerned some unpublished observations on these strains are therefore presented though not with any intention of "explaining away" our exceptions. Until further detailed analyses of the cancer record of these strains are completed and published it will obviously be impossible to decide whether or not they constitute real exceptions to the apparent rule found in other strains, of low chiasma frequency being associated with susceptibility and high with resistance.

Strain C 57 Black, the fifth strain to be examined, was the first strain reported as resistant which was found to have a low chiasma frequency. It is described as having "no recorded incidence of carcinoma of the breast . . . . . . during the last ten years." After its chiasma frequency had been found to be somewhat low (30.3) in the first test, this result was communicated to Dr. Little with a request for further information regarding the possible occurrence of other types of tumors in this strain. In reply Dr. Little stated

TABLE III

Differences in chiasma frequency in strains of mice

Necessary difference for P = 0.01 = 1.40

	CBA, 2nd test 31.72	CBA, 1st test 32.60	I, 33.92	N, 34.92	C 57 Black, 2nd test 29.72	C 57 Black, 1st test 30.32	M Leaden, 2nd test 26.68	M Leaden, 1st test 27.76	Carrel's, C 57 Black, 26.72	C 57 Brown, 27.88	Y Black, 29.16	Y Yellow, 30.00	D, 27.40	C-3-H, 29.16	A, 2nd test 29.24	A, 1st test, 28.44
A, 1st Test	3.28	4.16	5.48	6.48	1.28	1.88	1.76	.68	1.72	.56	.72	1.56	1.04	.72	.80	—
A, 2nd Test	2.48	3.36	4.68	5.68	.48	1.08	2.56	1.48	2.52	1.36	.08	.76	1.84	.08	—	—
C-3-H	2.56	3.44	4.76	5.76	.56	1.16	2.48	1.40	2.44	1.28	0.00	.84	1.76	—	—	—
D	4.32	5.20	6.52	7.52	2.32	2.92	.72	.36	.68	.48	1.76	2.6	—	—	—	—
Y Yellow	1.72	2.60	3.92	4.92	.28	.32	3.32	2.24	3.28	2.12	.84	—	—	—	—	—
Y Black	2.56	3.44	4.76	5.76	.56	1.16	2.48	1.40	2.44	1.28	—	—	—	—	—	—
C 57 Brown	3.84	4.72	6.04	7.04	1.84	2.44	1.20	.12	1.16	—	—	—	—	—	—	—
Carrel C 57 Black	5.00	5.88	7.20	8.20	3.00	3.60	.04	1.04	—	—	—	—	—	—	—	—
M, 1st Test	3.96	4.84	6.16	7.16	1.96	2.56	1.08	—	—	—	—	—	—	—	—	—
M, 2nd Test	5.04	5.92	7.24	8.24	3.04	3.64	—	—	—	—	—	—	—	—	—	—
C 57 Black, 1st Test	1.40	2.28	3.60	4.60	.60	—	—	—	—	—	—	—	—	—	—	—
C 57 Black, 2nd Test	2.00	2.88	4.20	5.20	—	—	—	—	—	—	—	—	—	—	—	—
N	3.20	2.32	1.00	—	—	—	—	—	—	—	—	—	—	—	—	—
I	2.20	1.32	—	—	—	—	—	—	—	—	—	—	—	—	—	—
CBA, 1st Test	.88	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
CBA, 2nd Test	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—

that only one malignant tumor, some sort of lymphoblastoma, had been reported in it at Bar Harbor during the last 10 years, but that Dr. Alexis Carrel had verbally reported finding internal tumors in 18–30% of the members of an inbred line developed from animals of strain C 57 Black which he obtained in 1928. When this latter sub-strain, "Carrel's C 57 Black" was later examined cytologically its chiasma frequency, as recorded above, was found to be very low—26.7. A second test of chiasma frequency in Little's C 57 Black gave a result (29.7) not significantly different from that of the first test (30.3). Recently Dr. R. Korteweg of the Antoni van Leuvenhoek Laboratory, Amsterdam, reported verbally to the senior author that in his sub-strain of C 57 Black, which was obtained from Bar Harbor about four years ago, he finds about 10% of spontaneous leukemia, but no tumors. Dr. Little has since kindly supplied the information that no cases of leukemia have, however, been reported in C 57 Black at Bar Harbor.

It thus appears possible that there may be some genetic difference between C 57 Black and the other three resistant strains, A, D, and C-3-H. It is the strain used in the crosses in which Murray and Little (45, 46) have shown that susceptibility is transmitted predominantly by the female parent. The publication of detailed records of the behavior of other resistant strains in crosses with susceptible ones will be awaited with great interest.

One other strain, M Leaden, reported as having "an extraordinarily low cancer incidence" was found to have a chiasma frequency as low as any of the susceptible strains. After the first test had shown a frequency of 27.8 this result was in turn communicated to Dr. Little who in reply informed us that it is derived from the same stock as the other exception. It originated as a mutant in 1929-30 from the C 57 Brown strain, which in turn was segregated in 1924 from C 57 Black which Dr. Little started inbreeding in 1921. In the experience of Dr. Joseph Murray, who, according to Dr. Little has had most experience with it, the M Leaden strain has an "extraordinarily low" tumor incidence, as stated in the published leaflet, but a number of tumors have occurred in old females. A second test of the chiasma frequency of M Leaden gave a result (26.7) even lower than the first.

Our results may therefore be summarized as follows: (1) four strains (or five if Carrel's C 57 is included) reported to have a "high incidence" of spontaneous tumors and one strain having "tumors at an advanced age" all have low chiasma frequencies; (2) three strains having "no tumors observed" in ten or more generations have high chiasma frequencies; (3) two related strains which though classed as resistant have, either at Bar Harbor or elsewhere, a small proportion of tumors or leukemia at an advanced age and which belong to a stock which has produced a susceptible strain, have intermediate or low chiasma frequencies.

Until further detailed records of the various strains both in pure line and hybrid studies are available it is doubtless futile to make further attempts to discover whether or not there is a consistent relationship between chiasma frequency and tumor susceptibility. If no spontaneous tumors whatever are found in strains N, I, and CBA, it appears that chiasma frequency may possibly give a more precise indication of genetic constitution in regard to cancer susceptibility—one less affected by environmental conditions and such factors as relative longevity—than direct tests. On the other hand, the discovery of tumors in these lines (or in sub-strains from them if unaccompanied by a characteristic change in chiasma frequency) would show that the present apparent general correlation lacks significance.

So far as present records go, there are, then, six susceptible strains with low chiasma frequencies, and three resistant with high frequencies, or a total of nine concordant cases, and there are two (related) discordant strains. Neither of the discordant strains is beyond suspicion with regard to its degree of resistance. This degree of concordance makes it highly improbable that the differences in chiasma frequency are caused by some unknown factor independent of tumor susceptibility or resistance, but does not, of course, entirely rule out that possibility.

If we assume for the present that there *is* a general correlation between low chiasma frequency and susceptibility to spontaneous occurrence of tumors, we may next consider its possible significance.* It seems to indicate, first, that in an organism susceptible to tumor formation the processes of chromosome "splitting", spindle formation and cytokinesis are not correlated normally or are near the borderline of lack of correlation. If so, then the condition of still more abnormal correlation found in tumor cells themselves may be due either to the loss of remaining regulatory genes or to their mutation. On any of the somewhat related, though in detail distinct, concepts of *(a)* orthogenetic gene variation, *(b)* premutation (cf. 24) or *(c)* genic "Grenzwerte" (47) the slight lack of correlation indicated by lower chiasma frequency would be due to the genes controlling and regulating mitosis being in part way mutated in susceptible strains. Or, control of mitosis may depend upon a number of genes and all but one of these be already mutated.

There are many cases of similar morphological changes being produced by either gross, readily visible, chromosome losses or deficiencies, and by "point" or gene mutations (neglecting as irrelevant for present purposes newer data on the nature of "point mutation"). Asynapsis also can be produced either by gene mutation or chromosome loss. It is therefore probable that if malignancy is due to somatic cell mutation it may be caused in some cases by gene mutation and in others by chromosome loss or deficiency. Mendelsohn (42), in a paper published since the above statement was first drafted, has shown that in both sectioned material and tissue cultures of cysticercus cysts of rat liver there are many abnormal mitoses. He concludes, "The onset of malignancy in the cysticercus cysts may be associated in some way with the presence of abnormal mitoses. Their presence seems to be a manifestation of a potential carcinogenic agent at work." Mustard and other non-carcinogenic irritants produced superficially somewhat similar inflammatory reactions, but no mitotic irregularities. This evidence, together with Mottram's (44) observation that certain carcinogenic agents cause chromosome irregularities, emphasizes the probable significance of chromosome mutation. Considering first this possibility: irregularities of mitosis might be expected to occur with greater frequency in susceptible animals than in resistant ones having a greater margin of safety in the correlation of these processes. Through irregularities of mitosis, chromosomes or parts of chromosomes bearing genes essential for the regulation of mitosis could readily be lost (see also 31). Carcinogenic agents or any "irritant" or chronic injury might be the immediate cause of these irregularities. On the other hand, if malignancy arises through point mutation, then, on any of the concepts mentioned above, the controlling genes in susceptible strains would much more readily mutate further to the ineffective state than would the comparable genes, well within the "Grenzwert" of resistant strains. It is possible that induced tumors (including here the cysticercus cysts) may originate predominantly through gross chromosome changes whereas "spontaneous" tumors may more often be due to "gene mutation". This is a speculation for which there is at present no direct evidence, but critical comparative studies of the chromosomes in both types of tumor from the same susceptible strain might be made to test it. The whole problem of the relationship, if any, between susceptibility or resistance to "spontaneous" tumors and reactivity to artificially applied carcinogenic agents is only just now receiving attention. Andervont (1) found that strains A, C-3-H, and CBA are all susceptible to the carcinogenic action of 1 : 2 : 5 : 6- dibenzanthracene, but that strains A, C-3-H, and also D (all susceptible to spontaneous tumor formation) react much earlier than do CBA and his stock mice, both of which strains "develop very few spontaneous growths". Lynch (1935) showed that seven different strains of mice all showed high incidence of sarcoma at the site of injection following subcutaneous injection of 1 : 2 : 5 : 6- dibenzanthracene. On the other hand the incidence of lung tumors ranged from 0.0 to 89.1% in the different strains and those that had the higher incidences were the strains that also have relatively high spontaneous occurrence of lung tumors. Without doubt there is a relationship between susceptibility to spontaneous occurrence of tumors and reactivity to certain carcinogenic agents, but the nature of the relationship is not yet clear in detail.

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* *See footnote on page 58.*

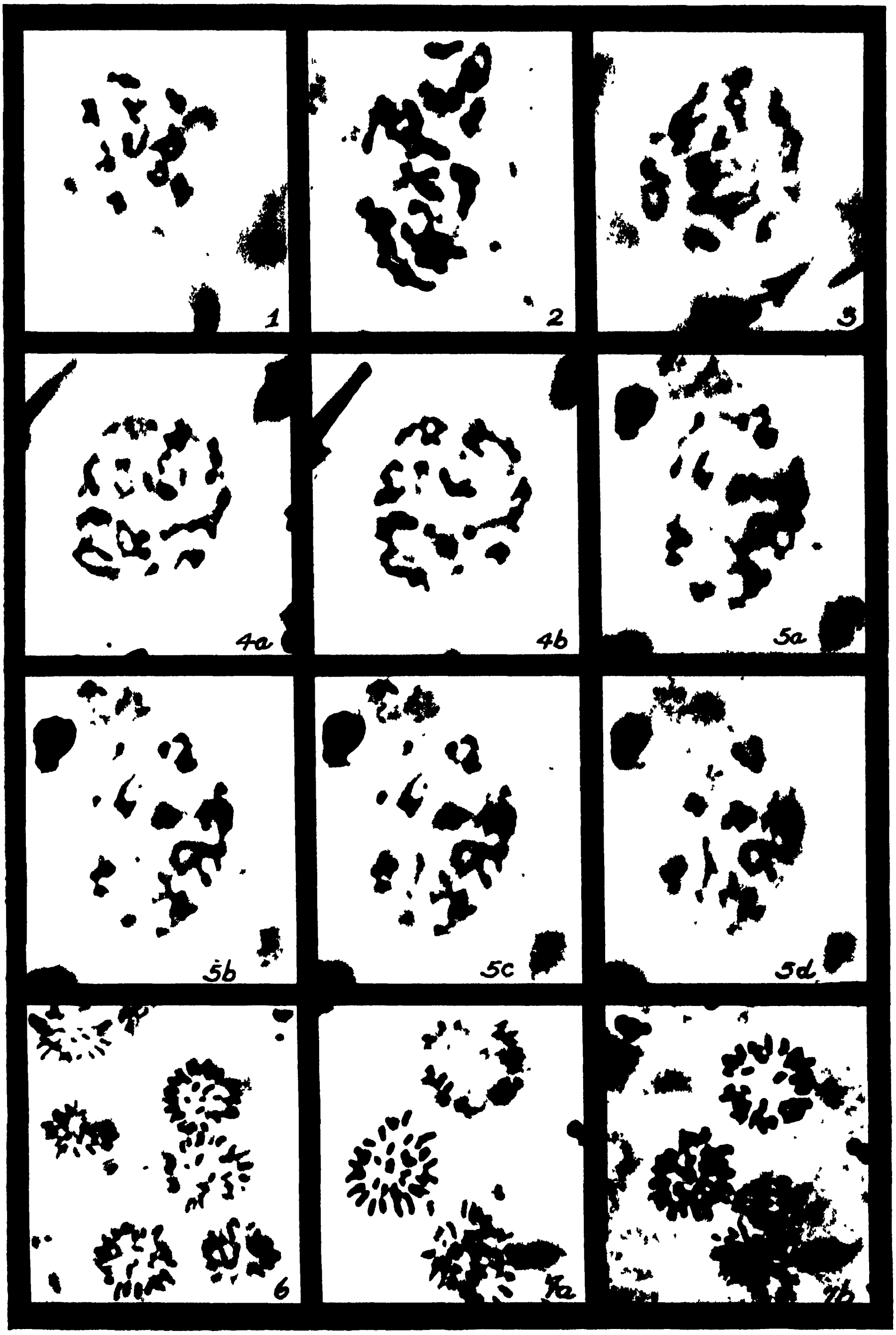
Lynch has shown also that susceptibility is organ-specific, and Potter and Richter (50) found that different lines of inoculated leukemias may, in susceptible mice, produce infiltrations in characteristically different situations.

If the correlation between chiasma frequency and tumor susceptibility is a general one, it almost certainly implies the existence of a generalized "cancer disposition". This concept is opposed by many leading workers in the field, who emphasize the specific nature and localization of tumors (or leukemias) in different strains. Without pressing the possible analogy, it may be pointed out that eversporting genes mutate with different frequencies in different tissues, and in the same tissues at different ages. The regular occurrence of malignancy in certain tissues may, however, be due to that tissue being the point of contact with the inciting stimulus, and hereditary susceptibilities for localization and specificity may be as indirectly related physiologically to the primary susceptibility gene as are the modifying genes in Bonnevie's mice (9) which influence the distribution of blebs and thereby the ultimate effects of the X-ray induced mutant gene which causes syndactyly, abortive development of the feet, etc. The work of Curtis, Dunning and Bullock (16) shows that in their case it is the immediate inciting cause which is localized, and that the nature of the tumor depends upon its region of origin. From the present data it is suggested that what is inherited in tumor susceptible strains is a mutated state for *most* of the genes necessary for the control of mitosis, or a heightened tendency of controlling genes to be lost, to mutate, or to pass the "Grenzwerte". The medium of transmission of susceptibility on all but the first of these suggestions may be either the cytoplasm or the chromosomes, or both. Murray and Little (45, 46) have shown that in the cross of Dilute-brown dba (susceptible) × C57 Black, susceptibility is transmitted predominantly in the female line, and therefore, they consider extra-chromosomally, but there is, they state, also evidence of chromosomal genes being involved. In a number of cases in plant hybrids chromosome pairing ("chiasma frequency") apparently depends on an interaction between the chromosomes and the cytoplasm—both may carry "factors" affecting it. Kostoff (32), by inducing polyploidy and consequent fertility in self-sterile *Nicotiana* hybrids, has shown that the tumors which occur in certain interspecific hybrids of this genus are heritable. Old plants have most tumors and in some cases the growing point may develop into tumors instead of branches. Kostoff notes that if tumors are due to somatic cell mutation, the mutation rate must be very high, and points out that some genes, for example that for red flower color, are known to be stable in the pure species in which they naturally occur, but highly unstable in hybrids. In species hybrids the paternal genes are of course in "foreign" cytoplasm. The danger of concluding that maternal transmission is necessarily plasmatic inheritance has, however, been strikingly shown by Dobzhansky (19).

The differences in chiasma frequency may, however, be only a symptom of differences in cellular metabolism, in which case the chromosome behavior is a primary concomitant of some underlying cause of tumor susceptibility. Since differences in cellular metabolism might possibly be revealed by differences in nucleolar size, studies of this were made, but we were unable to determine any difference in this respect between susceptible and resistant strains. Direct tests could, of course, be made where the necessary apparatus is available.

Resistance to the growth of implanted tumors or leukemias appears at present to be a completely distinct problem from the origin of neoplasms. That induced protection may be determined by the genetic constitution of the immunizing tissue is strikingly demonstrated by MacDowell, Taylor, and Potter (41) in an experiment of remarkable precision. As far as can be seen at present, however, this phase of the cancer problem is unrelated to the aspects of it that we are primarily concerned with in the present work.

PLATE I



Photomicrographs: FIGS. 1-5. *Diakinesis in primary spermatocytes, ca. 2000×.* FIGS. 6 AND 7. *Mitotic metaphases in spermatogonial cells, ca. 1500×.* FIG. 1. *C57 Black.* FIG. 2. *Carrel's derivative of C57 Black.* FIG. 3. *Strain I.* FIGS. 4A AND B. *M Leaden, successive focal levels.* FIGS. 5A, B, C AND D. *Strain A, successive focal levels.* FIG. 6. *C57 Black.* FIGS. 7A AND B. *Y, black, successive focal levels.*

Studies of somatic chromosomes in strains of mice susceptible and resistant to spontaneous tumors are being continued by the authors, at present independently, and by others in the hope that, with different techniques and tissues or different standards, it may be possible to assess on a more satisfactory basis the differences, if such are proved to be real, which at present can be referred only to a general impression. If these efforts are successful it may be possible to diagnose susceptibility by biopsy in advance of the occurrence of tumors. For the present, this study, being on spermatocytes, can have only indirect significance for the cancer problem in man. It is our hope that it may contribute to an understanding of the mechanism connecting hereditary transmission of tumor susceptibility and the origin of tumors.

Acknowledgments

Our thanks are due to Dr. C. C. Little, Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine, for supplying at nominal cost the mice used in this study and for giving us much unpublished information about them; to Dr. J. S. Potter, Carnegie Institution, Cold Spring Harbor, for helpful criticism and a reading of the manuscript; to Professor J. B. Collip, Department of Biochemistry, McGill University, for maintaining our mouse colony; to Professor E. W. Crampton, Macdonald College, for assistance with the statistical analysis; to the National Research Council of Canada for the grant of a fellowship to the junior author during the first year of this study (1933–4), and to an anonymous donor who, through Dr. E. W. Archibald, McGill University, provided a scholarship for the second year's work.

Appendix

A copy of the foregoing paper was submitted to Dr. C. C. Little, before publication, with a request for criticism and any new data available on the cancer history of the mice used. The data and information which Dr. Little supplied (January 21, 1936) are here appended rather than incorporated in the text for reasons which will become obvious in the presentation of them.

Strain C 57 Black now has two recorded instances of carcinoma of the breast. To the description of Strain Y may now be added that it has a low to medium incidence of breast tumors. One tumor has now been observed in Strain N. Strain C 57 Brown is now classed as having medium tumor incidence. All these are obviously minor additions to the records which do not change the general classification of the strains. A more serious alteration has occurred in the behavior of Strain CBA. From having for many years had no tumors of any description, it has "as the number of animals and number of generations of inbreeding increased" changed to the point where it must be considered as having a "low to medium tumor incidence." Dr. Little states that he and his co-workers have come to doubt whether there is any such thing as a cancer-free strain. As he says, "in view of our knowledge of the process of somatic mutation . . . . . . it would be surprising if sporadic mutation which might possibly give rise to a tumor did not occur." At the same time one wonders if the change in a strain such as CBA may not be due in part at least to segregation or mutation having changed its general constitution. In any case this change does not materially affect our general conclusions since CBA must still be classed as resistant. Further, all mice of Strain CBA cytologically examined were obtained from Dr. Little between October 1933 and April 1934, when it was still classed as tumor-free, which may be significant with regard to the question of whether a change has occurred in the strain. The chiasma frequency was 0.88 lower in the second test than in the first of this strain. The animal used in the second test was obtained six months later than the first. But even if this difference were considered as possibly significant, the fact that the count was made on a single animal and that one four months older than any in the first test, would prevent any conclusion being reached from it. It may, however, be worth while to make counts on young animals of this strain now.

Most significant for the present study are Dr. Little's comments on Strain C 57 Black. Of it he states: "We are getting fairly clear evidence that although C 57 Black does not itself give a high incidence of tumors, hybrids derived from it and other strains have in some cases given higher tumor incidence than would be expected if the strain C 57 Black was entirely devoid of genetic tendency to tumor formation." He notes also that Korteweg (1934, Nederl. Tigds. Geneeskunde 78 : 240-245) has shown it to react more positively to certain carcinogenic agents than the dilute brown strain does. There are thus further indications that our apparent exception in the correlation of low chiasma frequency with susceptibility may in the course of further work prove to be highly significant.

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**Even were the data entirely in agreement with expectation based on the initial working hypothesis (outlined briefly in the Abstract) that would not, of course, establish its validity. That it has enabled us to discover interesting differences in chiasma frequency is for the present sufficient merit whatever the ultimate decision regarding its validity as an explanation of the hereditary mechanism involved in cancer susceptibility may be. Says Bertrand Russell:*

"The fact is that it is difficult to think of the right hypothesis, and no technique exists to facilitate this most essential step in scientific progress. Consequently, any methodical plan by which new hypotheses are suggested is apt to be useful; and if it is firmly believed in, it gives the investigator patience to go on testing continually fresh possibilities, however many may have previously had to be discarded".

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STUDIES ON THE HETEROPHYID TREMATODE, *APOPHALLUS VENUSTUS* (RANSOM, 1920) IN CANADA

PART I. MORPHOLOGY AND TAXONOMY[1]

BY THOMAS W. M. CAMERON[2]

Abstract

This minute intestinal trematode occurs in the cat, dog, racoon and heron in the lower Ottawa Valley. Its morphology is redescribed and its nomenclature reconsidered. It is believed to be specifically distinct from the closely related form, *A. donicus* of Central Europe. *A. brevis* is recorded from the loon.

Introduction

The Heterophyidae is a family of very small Trematoda living in the small intestine of a wide variety of vertebrates. In recent years considerable attention has been given to this group as its members appear to show a very low degree of host specificity and to possess the ability of developing to functional maturity in any fish-eating bird or mammal, including the common laboratory animals. A considerable number of species has been recorded from man and most parasitologists believe that all are potential human species. Comparatively few life histories have been worked out, but in such as have, two intermediate hosts have been found to be essential—a snail and a fish.

The Heterophyidae live on the mucosa of the small intestine and until recently have been regarded as comparatively harmless worms. The recent investigations of Africa, Garcia and de Leon in the Philippines, however, have shown that, potentially at least, they may be very serious parasites indeed. These workers performed 108 almost consecutive autopsies in the Manila city morgue and discovered nine cases of intestinal infestations with Heterophyidae, four different species being involved. The cause of death in these nine cases was found to be cardiac trouble (six), malaria, lobar pneumonia and meningitis (one each). The high proportion of cardiac cases suggested some causal relationship with the parasites, and investigation showed that such was the case. The trematodes are characteristically covered with minute, backwardly directed spines; they live between the villi

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of the small intestine, and the spines must serve, in conjunction with body movements, to keep them in very close contact with the mucosa. Presumably land mammals are not "natural" fish-eaters, and therefore not so completely adapted to the flukes as are aquatic mammals and fish-eating birds, and so the trematodes may actually penetrate the deeper layers of the mucosa. The eggs, in some cases at least, are unable to pass into the lumen of the intestine, and because of their situation, gain entrance to the general circulation by way of the lacteals or venules of the villi, and are carried to various parts of the body. In the six cases mentioned, they had reached the smaller vessels of the heart where they had caused emboli, with fatal results.

The discovery of a member of the Heterophyidae common in the Montreal district, carried by fresh-water fish, is accordingly, of considerable interest.

In connection with the survey of internal parasites of animals in Canada, which is being conducted by the Institute, eggs of a species of Heterophyid—later found to be a species of *Apophallus*—were found to be common in cats in Ste. Anne de Bellevue, Quebec. With the permission and assistance of the Dominion and Provincial Departments of Fisheries, a large number of local fish was obtained and fed, by species, to non-infected cats, which had been bred at Macdonald College and which had not previously been fed on fish. In every case, with the exception of cats fed on Rock Bass, infections with the same species of *Apophallus* developed.

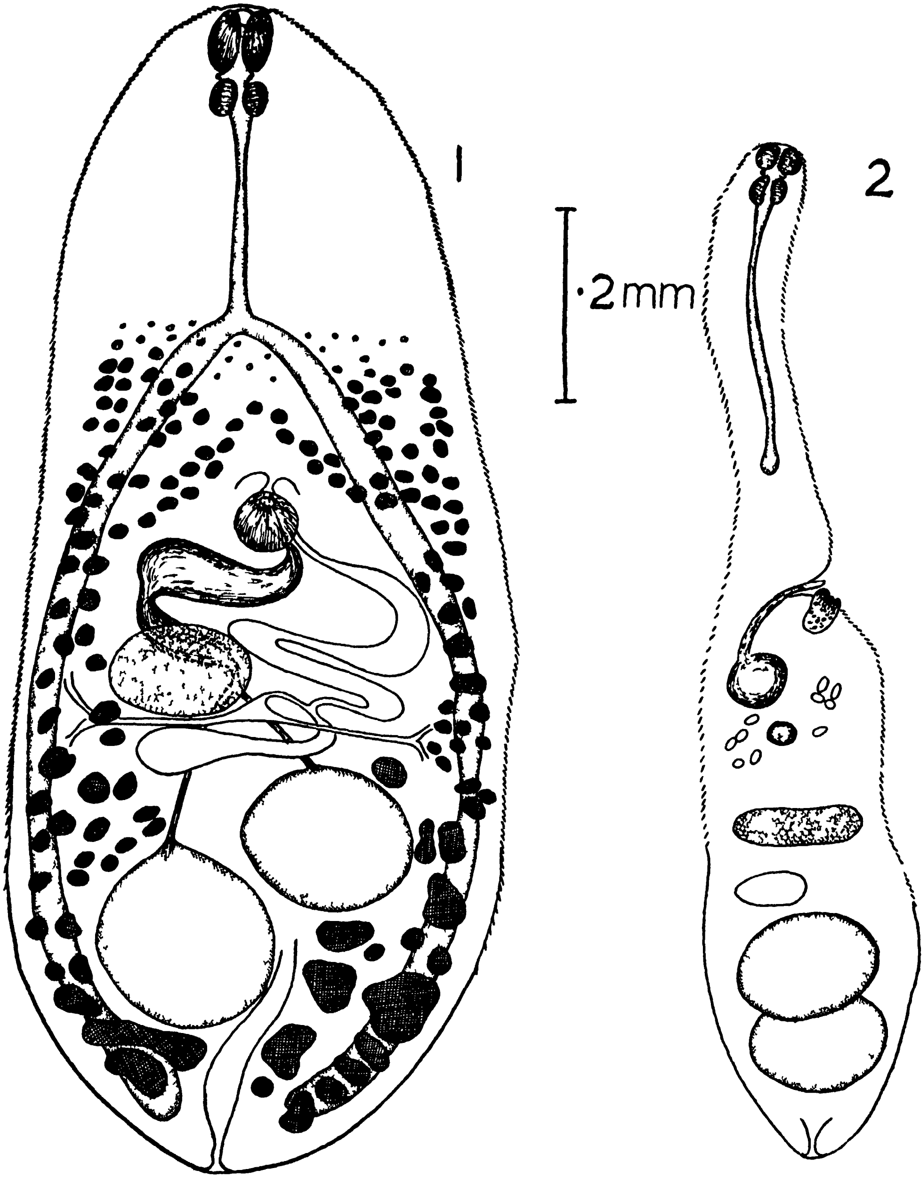
Adults ***Apophallus venustus*** **(Ransom, 1920)**

The contour of the body is elongated oval in most cases, being slightly broader at the posterior end (Fig. 1). In many cases however, it becomes narrower in the middle, resembling the sole of a child's shoe; in a few cases it is almost pyriform. These variations in outline are found in batches fixed, stained and mounted in bulk and so subjected to identical conditions of technique. It varies in length from 0.95 to 1.4 mm. and its maximum breadth is from 0.25 to 0.55 mm. The mean size is approximately 1.1 by 0.4 mm. The anterior half is only about half the thickness of the posterior half.

The cuticle is provided with numerous small spines in the anterior half. At the level of the genital pore they commence to decrease in number and the posterior quarter of the body is almost entirely free.

The oral sucker is slightly sub-terminal and ventral, and is oval in shape, measuring 0.07 mm. long by 0.06 mm. broad.

The ventral sucker is in the mid-line of the body just in front of the mid-point. It is slightly but distinctly muscular: in size, it is slightly smaller than the oral sucker (0.06 mm.). It is situated on the posterior margin of a genital sinus and is directed anteriorly. The genital openings occur at the base of the sinus, anterior to the ventral sucker, and from their antero-lateral margins arise two feebly muscular papillae (Figs. 1 and 2), the so-called genital papillae.



Apophallus venustus

FIG. 1. *Typical adult drawn from ventral aspect.*

FIG 2. *Medial view of adult, reconstructed from serial sections.*

Digestive System

The oral sucker is connected by a small prepharynx to the muscular pharynx. The oesophagus is long and simple and gives rise to two intestinal caeca, a considerable distance in front of the genital sinus and about a quarter of the body length from the anterior end. The caeca run to the posterior end of the body, terminating almost in contact with the excretory vesicle.

Male Genital System

There are two almost spherical testes, situated obliquely to each other in the posterior region of the body; the right testis is generally posterior. They have a diameter of 0.15 to 0.2 mm. The vasa efferentia unite at about the level of and dorsal to the ovary. The vas deferens quickly becomes enlarged to form a conspicuous seminal vesicle, which is roughly S-shaped with a central constriction and lies between the ovary and the genital sinus. It is continued by an ejaculatory canal to open in the base of the genital sinus on the same side as the ovary. There is no cirrus.

Female Genital System

The ovary is transversely ellipsoidal, measuring 0.15 mm. by 0.1 mm., with a smooth contour, and is situated anterior to both testes, on the same side of the body as the posterior one. Behind the ovary and towards the dorsal surface of the body, is a transversely oval spermatheca. Lying mainly on the side of the body opposite to that on which lies the ovary, but encroaching on the ovarian side of the body as well, is a relatively short uterus of several loops. It terminates at the base of the genital sinus, by the side of the male canal. Laurer's canal is absent.

The yolk follicles are numerous small groups of cells which occupy the posterior part of the body. Their anterior limit is about level with the junction of the two intestinal caeca, and is remarkably constant in all the specimens examined. The anterior follicles, which cross the mid-line of the body, are smaller and more numerous than the posterior follicles (Fig. 1.). The posterior follicles pass behind the testes and occupy all the otherwise unoccupied space there. The yolk ducts originate about the middle of the yolk glands, between the spermatheca and the ovary and unite to form a common yolk reservoir of varying size in the mid-line.

The eggs are few in number (up to 100) and measure 26 to 32μ by 18 to 22μ.

Excretory System

The excretory system was not studied in detail. The excretory vesicle is Y-shaped with the posterior stem S-shaped in order to pass between the two testes. The excretory pore is terminal.

Nomenclature

The systematic position of the various species in this genus has given rise, in the past few years, to a considerable amount of discussion.

The genus *Apophallus* was created in 1909, by Lühe, for a species from *Larus ridibundus* from Central Europe which had been misidentified in 1898 by Creplin as *Distomum lingua*, and renamed *Distomum mühlingi* by Jägerskiöld the following year. In the same year, Looss placed it in the genus *Tocotrema* and it was not until 1909 that Lühe made it the type of his new genus with the name of *Apophallus mühlingi* (Jägerskiöld, 1899) Lühe, 1909.

In 1920, Ransom (9) described a new species from *Larus delawarensis* (from Washington, D.C.) under the name of *Apophallus brevis* and created a new genus, *Cotylophallus* to receive two other new species, *viz:*— *C. similis* (from *Phoca vitulina* from Washington, D.C.) and *C. venustus* (from dogs, cats and *Vulpes lagopus* from the same area). These four species were separated from each other on the disposition of the yolk follicles.

Meanwhile, in 1919, Skrjabin and Lindtrop (10) created the genus *Rossicotrema* to receive the new species *R. donicum* from dogs and cats in Russia. This work was inaccessible to Ransom at the time of writing and there is little doubt that if he had known of it, he would not have created his genus *Cotylophallus* but would have included his species in the genus *Rossicotrema:* they were transferred to this genus by Ciurea in 1924.

In 1929, in a revision of the family, Witenberg (11), after examination of a·cotype of *A. brevis* Ransom, concluded that it also should be included in the genus *Rossicotrema.* He considered however that no specific differences existed among the four species now included in the genus and he suppressed all Ransom's species as synonyms of *R. donicum.* However, he recognized the genus *Apophallus* as distinct from *Rossicotrema,* mainly on the basis of the disposition of the testes, which were tandem to each other in the former and oblique in the latter. He regarded the genus *Tocotrema* as closely related to *Rossicotrema,* differing from it mainly in the presence of a single gonotyle (a name he applied to the genital papillae in these genera and analogous structures in other genera in this family), instead of a pair.

In the following year (1930) however, as the result of re-studying the available material, he concluded that the number of gonotyles was a specific rather than a generic character and he suppressed *Rossicotrema* in favor of *Tocotrema* (12). However, previous workers had suppressed the genus *Tocotrema* in favor of *Cryptocotyle;* Witenberg disagreed with this and recognized both genera as valid. This view has not been generally accepted and is especially criticized by Stunkard in his paper on the life-cycle of *Cryptocotyle lingua.* Witenberg had separated these two genera on the basis of shape of body and disposition of testes. Stunkard (1930) argued that the genera *Cryptocotyle* and *Tocotrema* were congeneric. He examined hundreds of *C. lingua* (which Witenberg had placed in *Tocotrema*) and found a few were as short and broad as *C. concava* (the type of *Cryptocotyle*), although ordinarily the body was longer and narrower. In a few specimens also, the testes lay at the same level, although normally they were diagonal. In *C. concava* however, the testes were on opposite sides of the median plane whereas in *C. lingua* both testes extended across this plane. He regarded these differences as specific rather than generic and referred both species to *Cryptocotyle.*

Price (7), in 1931, in a discussion on these species, concluded that *Cryptocotyle* and *Apophallus* were valid genera, separated from each other mainly in the structure of the ventral-sucker–genital-opening complex. In *Cryptocotyle* the genital sinus is a spacious, somewhat muscular structure, the ventral sucker is greatly reduced and is situated in the anterior wall of the sinus; the genital openings are behind the ventral sucker and the ducts open at the base of a *single* papilla-like gonotyle. In this genus he included *Tocotrema* Looss, 1899, as well as two other genera (*Hallum* Wigdor, 1918, and *Ciureana* Skrjabin, 1923) which are not discussed in this paper.

In *Apophallus*, the genital sinus is reduced in size and its walls feebly developed, while the ventral sucker, which is relatively strongly developed, is on its posterior margin; the genital openings are in front of the ventral sucker and two papilla-like gonotyles are present. In this genus he included *Rossicotrema* and *Cotylophallus*.

Four species of *Apophallus* were recognized by Price, *viz*:—

Body elongated, with more or less distinct constriction between ventral sucker and bifurcation of oesophagus:

Yolk glands extend to level of ventral sucker; oesophageal bifurcation about one-third body length from anterior end . . *A. mühlingi* (type).

Yolk glands do not extend anteriorly as far as ventral sucker: oesophageal bifurcation about one-fifth of body length from anterior end . . . . *A. crami* Price, 1931.
(from *Larus californicus*, Oregon).

Body ovoid in shape; yolk glands extend to level of oesophageal bifurcation or slightly beyond . . . . *A. donicum*
(including *venustus* and *similis*).

Body elongated pyriform in shape; yolk glands extend only slightly beyond ventral sucker . . . . *A. brevis.*

In the following year (1932) he described a new species, *Apophallus zalophi* from *Zalophus californianus* (8). This species is related to *A. donicus* but is smaller and thicker, has a much longer pre-pharynx and relatively large yolk follicles, which do not pass posterior to the anterior edge of the testes. There is, moreover, a greater tendency for the testes to be opposed. (Ciurea (6) (1933) has removed this species from the genus *Apophallus* to make it the type of a new genus, *Pricetrema.*)

Ciurea (1933) did not agree with Price in regarding the genus *Rossicotrema* as synonymous with *Apophallus*, believing the differences in the shape of the body, the situation of the testes and the development of the metacercaria, to be of generic importance.

Moreover, he did not regard *R. donicum* as being identical with *R. venustum* or *R. simile*. He believed all three species were valid and that they could be distinguished by the position of the anterior limit of the yolk glands. In *R. venustum*, these reach almost to the level of the bifurcation of the intestine; in *R. simile*, they pass this point, while in *R. donicum*, they do not reach it. The other species were left in *Apophallus*.

In 1935, Africa and Garcia (1) described *Apophallus eccentricus* from the dog in the Philippines. In this species, however, not only is the pre-pharynx long, but the genital pore is *lateral* instead of anterior, the yolk glands are considerably post-acetabular and the uterus extends to the posterior region of the body. This latter point alone seems sufficient to exclude it from this genus.

We have to deal with one of four possible species, *R. donicum, A. brevis, C. venustus* and *C. similis*, which have been variously referred to three gênera by modern authors, *viz.*:—

Witenberg (11, 12) (1929-1930) regards all four as synonymous and refers them all to the genus *Tocotrema* under the name of *T. donicum.*

Price (7) (1931) regards all four as belonging to the genus *Apophallus* considering the last two as synonymous with the first and so recognizing only two valid species, *A. donicus* and *A. brevis.*

In *A. donicus*, the body is ovoid and the yolk glands reach to the level of the oesophageal bifurcation, while in *A. brevis*, the body is pyriform with the yolk glands passing only slightly beyond the acetabulum.

Ciurea (6) (1933) regards all four as valid species and refers *A. brevis* to the genus *Apophallus* and the other three to the genus *Rossicotrema.* He separated these three species from each other as follows:

R. venustus—Yolk follicles reach almost to level of bifurcation of oesophagus.

R. simile—Yolk follicles pass in front of level of bifurcation of oesophagus.

R. donicum—Yolk follicles do not reach the level of bifurcation of oesophagus.

A considerable amount of this confusion is due to the fact that these three workers have adopted different generic criteria. Witenberg regards the relationship of the testes to each other as of fundamental importance; Price places emphasis on the anterior or posterior position of the ventral sucker relative to the genital openings; Ciurea considers not only the disposition of the testes, but the shape of the body and development of the trematode as important generic features, while considering the structure of the ventral-sucker–genital-sinus complex as of super-generic importance.

While Witenberg is possibly correct in regarding the presence of one or of two gonotyles as only of specific importance, it would seem that the opening of the genital ducts anterior or posterior to the ventral sucker is of very considerable importance, more so even than the relationship of the testes to each other. Accordingly, Price's criterion is accepted in this paper and *Tocotrema* regarded as a synonym of *Cryptocotyle*, and *Rossicotrema* as a synonym of *Apophallus.*

The question of species is much more difficult. There is a considerable similarity not only among the four "species" mentioned above, but among them and *A. crami* and *A. mühlingi.* They are differentiated on characters which appear to vary within wide limits.

Outline of Body

As mentioned above, the outline in most of my specimens is an elongated oval, somewhat more pointed at the anterior end than at the posterior, and slightly broader at the level of the testes than at the level of the oesophageal bifurcation. In a few cases, the body is markedly broader at the level of the testes and the body approaches the pyriform: in others the width at these two points is approximately similar, but there is a distinct constriction between them (Fig. 3). The majority are ovoid however, and the two exceptions do not reach the limits shown in Price's drawing of *A. crami*, or Ransom's drawing of *A. brevis*. It may well be the case that all the specimens of these two species have constant body outlines, but the fact that this is not the case in the Canadian material greatly reduces the value of this feature as a specific character.

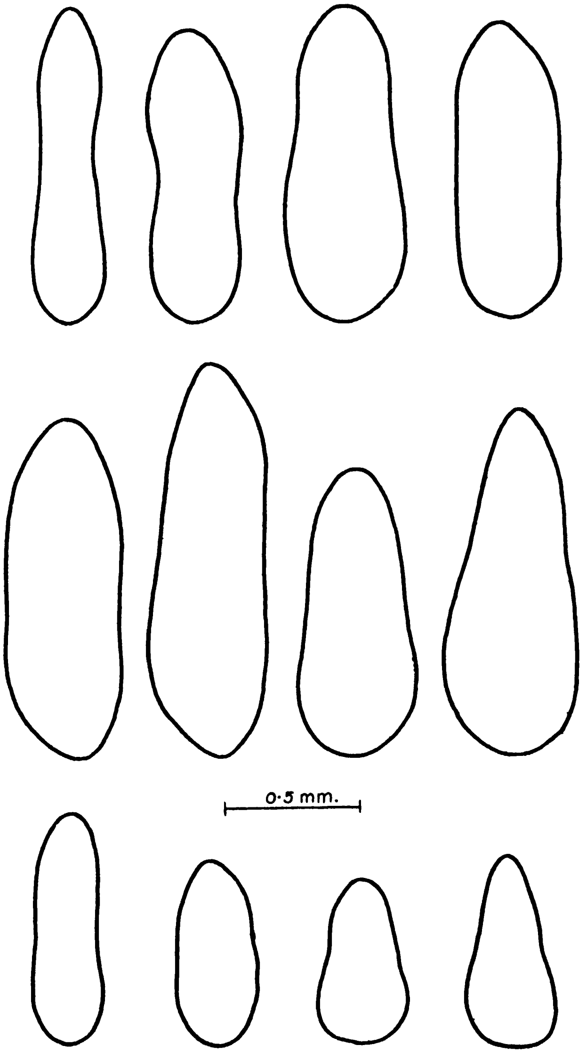


FIG. 3. *Camera lucida drawings, all to same scale, of A. venustus (upper two rows) and A. donicus (bottom row).*

Testes

In every case (except the obvious malformations mentioned later in this series), the testes are distinctly obliquely placed with regard to each other.

Yolk Glands

In all the Canadian specimens the yolk glands reach to about the level of the oesophageal bifurcation, which is placed about the junction of anterior and second quarters of the body. These yolk glands always meet across the middle line at this point.

There is a uniformity about all the drawings of *A. mühlingi* published. All have elongated forms, with the testes almost, but not quite tandem and the yolk glands ceasing at the level of the ventral sucker. In *A. crami*, the same elongated body form is found with the testes more obviously oblique and the yolk glands ceasing well posterior to the ventral sucker.

In *A. donicus* (in Ciurea's various drawings as well as in a number of specimens he kindly sent to me) there is considerable variation in body form (Fig. 3). The typical outline in expanded specimens is elongated oval, but contracted specimens are distinctly pyriform. In the former, the testes are oblique; in the latter they are almost side-by-side. However, the specimens show that the yolk glands terminate about the level of the ventral sucker and Ciurea also has drawn attention to this fact in his various papers on the subject (Plate 1, Figs. 1 and 2).

In *A. venustus*, on the other hand, which is otherwise similar to *A. donicus*, the yolk glands reach to the level of the oesophageal bifurcation (Plate I, Figs. 4, 5, 6). *R. simile* as described by Ransom, does not appear to differ specifically from this form, while *A. brevis* approaches very close to *R. donicum.*

I suggest dividing these species as follows:—

1. Yolk glands do *not* reach level of ventral sucker . . . . . *A. mühlingi.*
A. donicus.
A. brevis.

In this group, *A. mühlingi* has a long narrow body with the testes almost tandem; in *A. donicus* the body is oval to pyriform with the testes oblique; in *A. brevis*, the body is elongated pyriform and the testes oblique.

2. Yolk glands reach level of oesophageal bifurcation . . . . *A. venustus*
(including *C. similis.*)

Minor differences between *venustus* and *donicus* lie in the testes. In the former they are always distinctly oblique, more or less spherical and fairly posterior: in the latter, they tend towards a side-by-side arrangement, although generally distinctly oblique, are more or less triangular in outline and occupy a slightly more anterior position in the body. *A. donicus* has a maximum length of 1.14 mm. and width of 0.46 mm.: *A. venustus* has a maximum length of 1.4 mm. and width of 0.55 mm. The eggs of *A. donicus* are slightly larger than those of *A. venustus* (35-40 by 19–24μ and 26–32 by 18–22μ respectively).

Accordingly, I agree with Price in regarding *similis* as a synonym of *venustus*, but not in regarding *venustus* as a synonym of *donicus.* The specimens from Canada accordingly are referred to the species *Apophallus venustus.* As will be seen in the second part of this paper, there are certain biological differences between the American and European forms which further support this separation.

Apophallus venustus has only been recorded from eastern North America in Washington, D.C., and the lower Ottawa Valley, P.Q. It has been found in the following hosts: Dog, cat, racoon (*Procyon lotor*) Alaskan fox (*Vulpes lagopus*), harbour seal (*Phoca vitulina*) and the great blue heron (*Ardea herodias herodias*). The Alaskan fox came from the National Zoological Park in Washington City.

Apophallus donicus in the restricted sense used in this paper has been found in Europe from the following hosts, naturally or after feeding on infected fish: Dog, cat, rabbit, white mouse, *Vulpes lagopus* and *Mustela sarmatica.* It has also been recorded by Ciurea from the following birds: *Mergus merganser, Nycticorax nycticorax, Buteo buteo, Ciconia ciconia, Larus ridibundus, Sterna cantiaca* (the specimens in the two last-mentioned hosts were non-ovigerous), *Asio otus, Coturnix communis, Turtur communis* and *Columba livia* (the last four hosts mentioned were experimentally infected).

In addition to the specimens found in cats, a considerable number of specimens of *Apophallus* was recovered from a young loon (*Gavia immer*). The loon had only been a few days in the district and it is almost certain that its infection was not acquired locally. These specimens differ from the cat specimens in a number of points. The oesophagus is longer—about a third of the body length—and the yolk glands never reach to the bifurcation (Plate I, Fig. 3) but cease at the level of the ventral sucker. This point was quite constant in the 30 specimens examined. In all, the testes are obliquely placed to each other and are both rather more anterior than in the cat specimens.

There is considerable variation in the shape of the body, however. In some specimens this is elongated oval, but in most it lies between elongated pyriform and elongated with a more or less distinct constriction between the ventral sucker and bifurcation of the oesophagus. Using Price's criteria, some specimens could be called *A. mühlingi* and others, *A. brevis*. As however, in European specimens of *A. mühlingi* the testes are almost tandem to each other and in the present specimens they are always distinctly oblique, it would seem that it should more correctly be referred to *A. brevis.*

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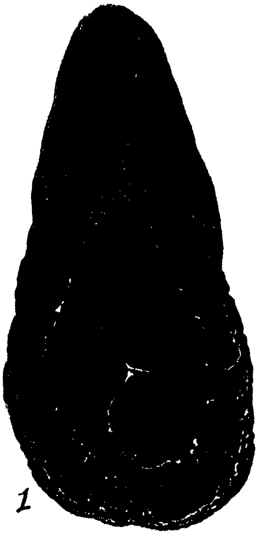
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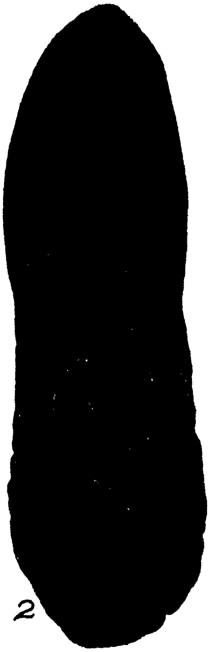
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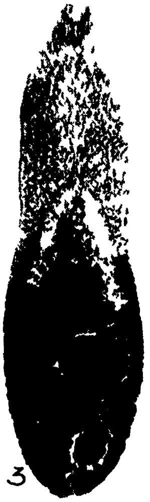
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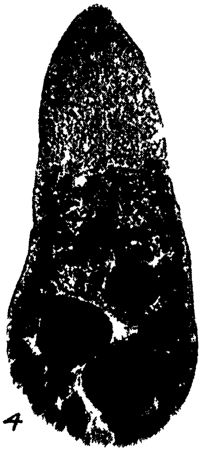
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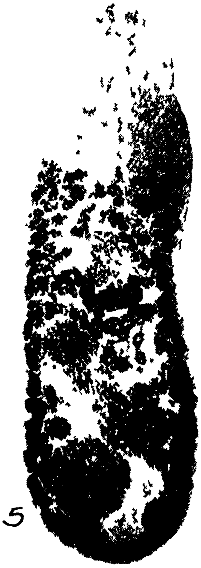
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FIGS 1 AND 2 *Apophallus donicus (from specimens from dog received from Professor Ciurea, Roumania)*

FIG 3 *Apophallus brevis (from loon Canada)*

FIGS 4–6 *Apophallus venustus (from cat, Canada)*

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STUDIES ON THE BIONOMICS AND CONTROL OF THE BURSATE NEMATODES OF HORSES AND SHEEP

II. TECHNIQUE[1]

BY I. W. PARNELL[2]

Abstract

This paper describes the standardization of the technique that has been evolved to facilitate the comparative testing of various substances that can be added to manure with the object of effectively destroying the free-living stages of bursate nematodes. Methods of controlling the experiments are described and the significance of the results discussed.

The unexpected lethal effects of urine on the eggs and larvae of bursate nematodes reported in Part I. of this series of papers (13) gave hope that other substances with a similar effect might be found, which could be added to feces and which would be of value in increasing the fertilizing properties of the manure or would be easily and cheaply obtained by the farmer. It also opened up the possibility of similarly treating human excrement in countries where hookworm disease is endemic and where lethal effect is more important than manurial accretion; a very large number of possible chemicals could be tested for this purpose. Moreover, further tests on urine were necessary. This made it essential that a standardized technique should be evolved, not only for the actual tests but for the large number of controls necessary. The present paper describes the technique that was adopted and the methods that were used to control the experiments. Future papers will describe further experiments with urine, with nitrogenous and with non-nitrogenous artificial fertilizers, with various chemicals used to control plant eelworms and insects, with disinfectants and with some other miscellaneous chemicals which appeared to have potential applications.

To enable comparisons to be made between the different substances tested, the technique already described in Part I (13) of making the cultures in cheese cloth which could be suspended in funnels for the extraction of the larvae, has been used throughout. This method of culturing the feces in a cheesecloth bag allows excellent drainage, yet the lightly covered sealer retains sufficient moisture for many weeks and contains sufficient oxygen for development of the larvae. Maplestone (11) has pointed out that it is not necessary to trap hookworm larvae if they are allowed to develop under suitable con-

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ditions, and that Lane's (7) traps are only necessary when there is bad drainage, such as in a Petri dish, which causes unsuitable conditions from which the larvae migrate. Sclerostome larvae in the sealers have seldom been seen as high as the neck, and few, if any, reach the lid, although it, as is the sealer, is always washed into the funnel.

All cultures have been made from horse feces collected in a single stable of adult heavy-draft horses, all on a similar diet; when feces from more than one horse have been used or when many cultures were made at the same time, more than one "control" has been made.

All cultures have been kept in a dark constant temperature room at 25° ± 2° C., until the larvae were extracted, except those treated with chemicals that give off gases that are undesirable in a small unventilated room,—chloropicrin, carbon disulphide and calcium cyanide.

The cultures were always made of 40 grams of horse feces weighed when fresh, but no allowance was made for any variation in the consistency of the feces. The variation, owing to the uniform treatment of the horses, was slight. The cultures were always kept for more than nine days to ensure that all larvae had time to reach the infective stage, and be acted upon in that stage. Usually they were kept considerably longer. Lucker (8, 9) Ortlepp (12), Taylor (16), and others have shown that the infective stages are reached in less than a week at temperatures similar to or lower than that at which these cultures were kept.

Two methods have been used to test the action of the fertilizers on eggs or larvae or both in the presence of feces. The method used in treating the cultures to be discussed in the papers that will follow, has been to allow the chemicals to remain in the cultures so that they could act on all stages subsequent to the incorporation of the chemicals. The other method to test the action of the chemical on eggs and free-feeding larvae only, and not on subsequent stages, has been to treat the feces either while fresh (and the eggs therefore undeveloped) or after there has been time for the appropriate amount of development. After the chemical has been in the feces for a sufficient length of time, *e.g.*, 24 or 48 hours (during which time the majority of the larvae would develop to the appropriate stage) the greater part of the chemicals has been removed by washing the cultures in several hundred cubic centimetres of warm water, squeezing them until there was only sufficient water for optimum development, filtering the washing water, and culturing the filter paper in the same sealer as the feces.

It is hoped that a comparison of the various results obtained when incorporating the same quantities of chemicals will demonstrate the most practical stage at which to treat feces with each of the more lethal substances.

When the egg stage, with or without the succeeding stages, has been included in the "treated" period, the feces have been mixed with the chemical, fertilizer, or other substance to be tested, within four hours of being passed.

Solid fertilizers or chemicals have been tested by mixing dry with the feces, and also as solutions if they are soluble in water. Sometimes "solutions" have been used in strengths above the point of solubility, but for the purposes of these papers these suspensions and solutions are all referred to as solutions. Fluids have been tested undiluted, and also diluted if miscible with water. It will be seen from the tables that in testing any fertilizer or other substance, the cultures were made up with the chemical and feces in a wide range of proportions. Even when these had indicated the lethal percentage necessary, confirmatory tests were made not only with the indicated proportions but also in greater and smaller amounts; the important range was tested in triplicate, the quantities above and below in duplicate. When desirable, cultures were also made well above or below the lethal point, not only for confirmation but for comparison with other fertilizers or chemicals. Unfortunately, the supply of urines, especially stale urines, prevented them being tested quite as thoroughly.

Twenty-five cubic centimetres is more than sufficient to moisten thoroughly 40 grams of fresh horse feces, and with this quantity of fluid, and sometimes even with 20 cc., there is some drainage from the bag of feces to the bottom of the sealer, so that part of the larger quantities of fluid used is only temporarily in contact with the Sclerostome eggs.

Excessive moisture may assist in the prevention of the development of eggs and free-feeding larvae in the centre of a mass of feces, by excluding the oxygen necessary for development. Moisture also alters the bacterial and mycological flora of the feces; the former may be an important food of free-feeding larvae, the latter may be parasitic on them. This flora may also be altered by some of the chemicals tested.

Fungi have been reported which are parasitic on nematodes and which use a three-dimensional net-like structure to capture their prey. Others, described by Drechsler (1, 2) and Sherbakoff (14), capture their prey in constricting loops, or by means of adhesive material on globose protuberances, after which the mycelium grows into the nematodes. It is probable that these fungi, under suitable conditions, may materially reduce a nematode population.

Occasionally the feces were contaminated by free-living nematodes before collection, and with some fertilizers these persisted in cultures in which the Sclerostomes were killed, and so the results are not applicable to free-living species. In a few cases the free-living species may have been predators, but contamination has not occurred sufficiently frequently to influence the conclusions.

To determine the number of larvae which reached the infective stage, the cultures were put into glass funnels, closed at the bottom by a clip on rubber hose. The inside of the sealer was washed into the funnel to collect all larvae which had migrated from the culture, and the funnel filled with warm water. In the funnels the larvae were stimulated to leave the cultures by scooping off or pouring off the top fluid and adding an equivalent amount of hot water nearly every day. Large funnels were used during the extraction in an endeavor to prevent the chemicals acting on the larvae during their collection. Pouring off the top fluid several times during the process has three major advantages:—

(1) It dilutes very considerably any chemical that has been used to treat the feces.

(2) The added hot water stimulates the larvae.

(3) Emptying and filling the funnel dislodges any larvae on the sides of the funnel more effectively than tapping the funnel, as described by Lane (7).

It has the disadvantage that it washes down any sediment, which would be serious in soil cultures, and thus has added to the difficulty of counting, especially in cultures treated with lime and phosphate and some other chemicals such as cyanamide.

While some dead Sclerostome larvae may float—Hirst (4) has found that hookworm larvae do—the great majority sink, unless supported by the side of the container or by floating foreign matter. Therefore this technique does not lose dead Sclerostome larvae which are outside the culture.

To estimate the number of larvae extracted from a culture the bottom fluid was drawn into a 50 cc. Erlenmeyer flask in which the larvae were left to settle; the top fluid was then discarded and the larvae at the bottom were poured into a square Petri dish which was divided into squares. In dealing with negative cultures or with cultures containing a few to two or three hundred larvae, the whole dish was examined. When there were only a few thousand larvae their numbers were estimated by counting those on a known area of the dish, always at least one sixth of the area. When there were several thousand larvae their number was estimated by shaking them up in 40 cc. or 80 cc. of water, and counting the number in 10 cc. or 20 cc.; in this case, one larva in at least every 24 was counted. When the larvae were exceptionally numerous, *i.e.*, 50,000 or more, the fluid was usually diluted in the same proportions again, in which case the number counted multiplied by 96 gave the number of larvae in the cultures. No culture was considered negative until no larvae could be collected after it had been in the funnel for more than four days at least. Positive cultures were kept in the funnels until the larvae that could be extracted were only a negligible proportion of those already counted; in a few cases this was two or three weeks, since some substances appeared to immobilize rather than kill the larvae. In a few cases, after having been in the funnels for some days, and the majority of the larvae having apparently been extracted, the cultures were broken up and rebagged to ensure that the majority of larvae had left the centre of the culture. More larvae were obtained from the cultures under these circumstances, but never sufficient to alter the significance of the number of those originally collected.

The counting of Sclerostome larvae presents an important difference from the counting of eggs, because the larvae when on a microscopic stage, under the influence of a fairly bright light, become very active; this not only makes them more difficult to count when numerous, but may involve their being counted twice or missed, when they swim from one row to another. Furthermore fungi or debris occasionally make distribution of the larvae throughout the dish most unequal, since the larvae may very rapidly become caught and form large struggling masses. Therefore, in spite of the fact that the larvae in the cultures have been estimated by carefully counting a large proportion of them and all the results have been checked, the numbers given must be considered as only approximate when the larvae were numerous. However this does not obscure the object of the work—to show the proportion of the various fertilizers or chemicals necessary to kill the free-living stages of Sclerostomes in feces and to suggest their relative strengths as sterilizing agents against the bursate nematode larvae.

To prevent a false impression of the accuracy obtained, the tables in these papers show the number of larvae isolated on the following basis:

1- 10—Number counted.
10- 20—Even numbers
20- 40—Nearest number divisible by 5
40- 60—Nearest number divisible by 10
65- 200—Nearest number divisible by 25
200- 750—Nearest number divisible by 50
800- 4,000—Nearest number divisible by 100
4,000- 8,000—Nearest number divisible by 200
8,000-30,000—Nearest number divisible by 500
Over 30,000—Nearest number divisible by 1,000

In a few cases, especially with active larvae or where there was much debris, even these figures may suggest an unjustifiable accuracy.

Little significance need be placed on the fact that in some cultures a few larvae are found, since when the cultures are tied up in cheese cloth and put into sealers, it is difficult to prevent minute pieces of feces and the eggs therein from adhering to the outside of the culture away from the influence of the fertilizer, or some feces and eggs from being squeezed on to the neck of the sealer when the culture is being put into it. Contamination of the counting dish or of the funnel is also a possible cause of an occasional culture not being completely negative when it should be, since the only glassware it has been possible to sterilize has been the sealers; the funnels, flasks and counting dish have only been washed. Another cause may be that the feces contain only a few eggs of a species resistant to the effects of the chemical being tested. No attempt has been made to determine the percentages of the various species of larvae, but the writer's impression is that the percentage of the *Strongylus* spp. has been lower than that in cultures made by Mahlau (10) in Berlin. It has been noted frequently that the larvae of *Strongylus* spp. are more resistant than those of *Trichonema* spp. to unfavorable conditions, whether chemical or physical.

In the tables showing the effects of the various substances on the numbers of larvae, a letter is used to indicate the condition of the larvae as follows:—

a—Most or all larvae dead.

b—A considerable proportion of the larvae dead.

c—A few of the larvae dead.

d—Much larval debris uncounted.

Although the larvae may have looked perfectly healthy it is impossible to say whether or not they would have been capable of infecting horses. It has been found that when only a few larvae survived in a culture, their life in water in the laboratory tended to be shorter than those from cultures in which many larvae survived, apart from the fact that if an excessive number of larvae are kept in a small quantity of water, they die fairly rapidly.

Each number is followed by a Roman numeral, which corresponds to the number of the control or controls listed in the table of controls for each paper; the same numeral is used for the same control in all the papers, so that not only can the significance of the number be appreciated, but cultures made from the same series can be traced.

The time between the making of the cultures and the extraction of the larvae is shown in weeks or fractions of weeks. The date on which the cultures were made is also shown, since Hornung (6) and Wagner (17) found that the egg count of horses in Germany varies with the season, although the observations of Gogolka (5) are to the contrary.

Tests have been made to determine the probable natural accuracy or inaccuracy of parts of the technique used to prevent any undue significance being attached to the variations in the numbers of larvae obtained.

To determine what significance could be put on the numbers of larvae obtained from untreated cultures, egg counts were made of feces from three horses on two consecutive days, the Stoll egg-counting technique (15) being used. At the same time 20-gm. cultures were made up and after 15 days these were baermanned. Four weeks later similar egg-counts and cultures were made with feces from the same horse; the results of both are shown in Table I.

TABLE I

NUMBERS OF LARVAE OBTAINED FROM CULTURES OF UNTREATED HORSE FECES SHOWING THE NUMBER AND PERCENTAGE OF LARVAE OBTAINED, COMPARED WITH THE ESTIMATED EGG CONTENT

Horse	Day	Number of larvae	Percentage of estimated number of eggs reaching the infective stage
Bay	1st	13,500	57
Bay	2nd	16,000	44
Bay	29th	6,600	14
Bay	30th	26,500	56
Roan	1st	13,500	45
Roan	2nd	12,500	40
Roan	29th	6,600	26
Roan	30th	9,000	43
Grey	1st	6,200	39
Grey	2nd	7,200	37
Grey	29th	2,200	55
Grey	30th	6,000	60

Feces were collected from another horse on two consecutive days, to determine whether there was any significant variation in the numbers of larvae obtained when the feces were cultured broken up or in lumps, and whether there was any significant variation in the numbers of larvae obtained from the feces first passed and those last passed from the horse. On each day, five 40-gm. cultures were made. One culture was made of one large lump, another was partly and a third completely broken up, using the feces that were passed first. From the feces passed last one culture was made of one large lump and another of completely broken up material. The larvae were isolated after culturing for 31 days. Table II shows the numbers of larvae obtained.

TABLE II

NUMBERS OF LARVAE OBTAINED WHEN THE FECES ARE BROKEN UP OR NOT BROKEN UP BEFORE CULTURING, AND THE NUMBERS OF LARVAE OBTAINED FROM THE FECES FIRST PASSED COMPARED WITH THOSE LAST PASSED

	In lumps		Partly broken up	Broken up	
	First passed	Last passed	First passed	First passed	Last passed
1st day	14,500	71,000	43,000	73,000	39,000
2nd day	37,000	44,000	57,000	41,000	45,000

Average per gram: In lumps, 1,040; Broken up, 1,240; First passed, 1,106; Last passed, 1,244.

To determine the efficiency of the larval extraction method nine cultures were twice sterilized by heat, (steam and dry), after which 75 to 100 larvae were poured into three cultures, 700 to 1,000 into three others, and about 10,000 larvae into three more. The larvae were then extracted from these cultures in the ordinary way. From the three cultures which received the smallest number of larvae 12½%, 25% and 45% were recovered; from the three cultures in the second group the recovery was 35%, 35% and 50%; and from the one containing about 10,000 the recovery was 45%, 65% and 90%. All the larvae used were from control cultures previously baermanned, the three 10,000 lots having been extracted a few days before being put back into feces, while the others had been collected a day or two earlier. There is a considerable tendency for larvae to become less active after they have been in water for a few days, unless affected by some stimulus such as a bright light; therefore a smaller percentage than usual would be recovered. Hirst (3), working with hookworm larvae added to various soils, found that approximately 12% were recovered in 24 hours from laterite soil, 17% from fine sand and 82% from gravelly loam with much humus.

To test the effects of the addition of water on the number of larvae obtained from 40 gm. of horse feces, various quantities were added to otherwise untreated fresh feces. Table III gives the number of larvae obtained. This table suggests that the addition of the larger quantities of water decreases the number of larvae which survive.

To supplement Table III details of the results obtained with rabbit urine, "byre fluid" etc. will be given in full in Paper III instead of a brief report of the negative results obtained with them.

TABLE III

QUANTITY OF WATER ADDED TO 40 GM. OF FRESH HORSE FECES, AND NUMBERS OF INFECTIVE SCLEROSTOME LARVAE ISOLATED AFTER CULTURING

	1.0 cc.	2.0 cc.	3.0 cc.	4 0 cc.	5 0 cc.	7.5 cc.	10.0 cc.	15.0 cc.	20.0 cc.	25 0 cc.
	22,000	30,000	22,500	23,000	8,500	20,000	17.000	15,000	3,600	5,200
	5,000	49,000	43,000	47,000	38,000	24,500	37,000	33,000	15,500	900
	38,000	39.000	24,000	4,700	49,000	16,000	18,000	19,000	16,000	7,000
		10,500	40,000	35.000	31,000	5,400	45,000	25,000	20.500	20,000
		24,500	31,000	23,500	3,500	1,800	13,000	1,100	10,000	3,500
		900	26,500	6,600	22.000	27,500	31,000	15,500	5,000	22,500
		12.500	23,500	22,000	9,000	42,000	6,800	17,000	22,500	33,000
		43,000	39,000	36,000	13,500	22,500	4,000	25,500	32,000	13,500
		42,000			30,000		9,000	52,000	15,500	8,000
		53,000			42,000		40,000	5,800	35,000	26,500
					17,000		34,000	15,000	27,000	2,400
					62,000		30,000		12,000	19,000
							13,000			27,500
										13,000
										39,000
										10,000
										3,700
										3,500
										7,800
Average no. of larvae isolated from cultures to which water was added	21,500	30.000	31,000	24,500	27,000	20,000	23,000	20,500	19,000	14,000
Average no. of larvae isolated from the untreated control cultures	39,000	27,000	29,500	29,000	30,000	32,000	25,500	24,500	37,000	28,000

In addition to the results tabulated, one culture treated with 0.5 cc. of water yielded 12,500 larvae, compared with 13,000 in the control; two treated with 12.5 cc. of water yielded an average of 36,500 compared with 47,000 in the controls. Twelve treated with 10 cc. water, including six in which chaffed sterilized straw was incorporated, but for which no dry controls were made, yielded an average of 21,000 larvae, while six similar cultures treated with 15 cc. of water yielded an average of 11,500 larvae.

TABLE IV

RATE OF COLLECTION OF LARVAE DURING THE ISOLATION PROCESS

Time hr	9 in funnel %	8 in funnel %	6 in funnel %	Remarks	Average of the 3 funnels %	Average rate per hour, %
1	50.0	48.2	42.55	Natural and artificial light	46.75	46.75
2	11.11	13.4	8.5		11.15	11.15
3	2.22	5.54	2.55		3.67	3.67
4	2.22	2.77	1.49	Hot water added to funnels immediately after 4th hour	2.06	2.06
5	6.53	4.06	9.7		6.53	6.53
6	8.88	8.93	4.84		7.53	7.53
6–8	5.83	4.3	1.54		3.76	1.88
8–10	0.83	1.47	0.96		1.13	0.56
10–12	0.35	0.4	0.69	Dark	0.49	0.25
12–14	0.11	0.4	0.48	Artificial light	0.35	0.17
14–16	0.28	0.4	0.42	Dark	0.38	0.19
16–20	0.17	0.36	0.64	Artificial light	0.4	0.1
20–24	0.35	1.3	1.27	Natural and artificial light	1.04	0.26
24–28	0.22	0.58	1.17	Immediately after the 28th hour the top water was poured out of the funnels and hot water added	0.69	0.17
28–32	1.4	0.98	12.72		5.07	1.27
32–36	0.49	0.89	1.22	Artificial light	0.9	0.22
36–42	0.11	0.27	0.42	Dark	0.28	0.05
42–48	0.01	0.36	0.1	Natural and artificial light	0.18	0.03
48–54	0.06	1.07	0.26		0.54	0.09
54–60	3.75	0.85	0.37	Immediately after the 54th hour the top water was poured out of the funnels and hot water added	1.44	0.24
60–72	0.65	0.57	0.37		0.51	0.042
72–84	1.53	0.89	1.1	A similar water change was made after the 72nd hour	1.1	0.09
84–96	0.21	0.4	0.96	Dark for some hours	0.54	0.045
96–108	0.17	0.18	0.19	Natural and artificial light	0.18	0.015
108–120	0.17	0.13	0.26		0.19	0.016
120–132	0.83	0.27	0.64	Water changed immediately after 120th hour	0.54	0.045
132–144	0.08	0.18	0.42	Dark for some hours	0.24	0.02
144–156	0.42	0.27	0.48	Natural and artificial light	0.38	0.032
156–168	0.08	0.13	0.42		0.22	0.018
168–192	0.14	0.05	0.53	Water changed at 174th and 178th hours	0.24	0.01
192–216	0.17	0.18	0.42	Cultures broken up and rebagged to release any imprisoned larvae at 193rd hour Hot water added at 195th hour.	0.26	0.011
216–240	0.25	0.4	0.42	Water changed at 216th and 227th hours	0.37	0.015
Total number of larvae.	35,900	56,000	47,100			

To determine the rate at which the larvae were extracted from cultures, three "control" cultures which were kept in the constant temperature room for 18 days, were drawn off at frequent intervals—hourly for the first six hours, every two hours for the next ten hours, every four hours for the following 20 hours, every six hours for the next 24 hours, then every 12 hours until the cultures had been in the funnels for a week. During this first week, except for the frequent drawing off of fluids, the cultures were treated in the usual manner, *i.e.*, the top waters were changed at the usual intervals, the lights were on in the room in which the funnels were kept for the usual periods, etc. During the three days after the end of the week everything was done to ensure that the larvae were extracted; hot water was frequently added, the cultures were broken up and rebagged with the centre on the outside, and lights were continually on. The larvae were counted every 24 hours.

The cultures were extracted in a nine-inch, an eight-inch and a six-inch funnel: they were first put in the funnels at ten o'clock in the morning.

Table IV shows the rate at which the larvae were extracted.

Where the larvae were numerous, the percentage, to two places of decimals, gives a false impression of accuracy, but two places of decimals are necessary when only a few were extracted.

The tests on the accuracy of the technique that has been employed, show that while large variations in the number of larvae isolated from controls containing numerous larvae have little importance, the utmost significance can be put on negative cultures.

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FURTHER STUDIES ON *FASCIOLOIDES MAGNA* (BASSI, 1875) WARD, 1917, AS A PARASITE OF RUMINANTS[1]

BY W. E. SWALES[2]

Abstract

Comparative studies have been made on the tissue reactions in fascioloidiasis magna in *Bos taurus*, *Bison bison*, *B. taurus* × *B. bison*, *Cervus canadensis*, *Odocoileus virginianus* and *Ovis aries*. Data have been obtained that support the hypothesis that the Cervidae are normal hosts in the trematode's life-cycle, that the large Bovidae do not act in this capacity and that *Ovis aries* may act as a definitive host but is severely injured by even light infestations. The cellular bases of the defence reactions in the above ruminants are recorded.

Since the publication by the author (10) on the life cycle of *F. magna* and on the preliminary findings in regard to the histopathology of fascioloidiasis magna, further work has been done concerning the parasite in the final host. Krull (7), in a series of experiments involving the experimental infestation of rabbits, sheep, cattle and a pig, with encysted cercariae from *Pseudosuccinea columella*, has corroborated and extended the preliminary observations. In view of Krull's work it seems advisable at this time to present the results of a series of studies made in an attempt to verify and explain the abnormal host-parasite relationships from immunological and histopathological points of view.

As previously shown, the parasite is very pathogenic in *Ovis aries* owing to its almost unchecked migration in the liver tissue. In large Bovidae, *viz.*, *Bos taurus*, *Bison bison*, *Bos grunniens* and *B. taurus* × *B. bison*, the parasite is completely enclosed in a fibrous cyst before it reaches sexual maturity, hence the life cycle is interrupted and these ruminants are unable to act as normal definitive hosts. Also the early cessation of migration of the parasite in large Bovidae prevents undue tissue destruction, resulting in a comparatively low pathogenicity.

In the Cervidae studied, *viz.*, *Cervus canadensis*, *Odocoileus virginianus*, and in other species of Cervidae observed by other workers and myself, the parasite is able to migrate in the liver tissue for some distance before becoming encapsulated. It then becomes enclosed in an adventitious fibrous cavity which differs from the cyst formed in large Bovidae in that it is comparatively thin-walled and most of the intercepted bile ducts are not occluded. These bile ducts then become afferent and efferent ducts in relation to the cavity, the

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former carrying bile from the liver tissue and the latter carrying away the ova and vomitus of the trematode into the hepatic duct, whence they reach the alimentary canal of the host. Thus in Cervidae a host-parasite balance is reached, the parasite being able to continue the life cycle, and the host is not unduly injured by any but very heavy infestations.

The above hypothesis needs the support of further observations upon numerous infested specimens of *Bos taurus*, even though the published works of Krull and the writer at least appear to indicate the rule. In addition to these data are the following observations made upon the sheep (*O. aries*, No. 3) which was still living at the time of my previous publication, and upon an experimentally infested calf (*Bos taurus*).

The sheep (Plate I, Fig. 1) was killed and injected on the 206th day of infestation; at that time it appeared to be near death from fascioloidiasis magna, being extremely cachectic and unable to stand. Eighteen weekly fecal examinations made by a technical assistant failed to reveal the presence of ova of *F. magna*. The autopsy revealed a liver of normal size which was firmly adherent to the diaphragm. Allowing for the change due to the injected fixative, the organ was pale in color, but several areas showed small pigmented spots. An abscess-like projection was present on the left side near the impression of the vena cava. The gall bladder was distended and contained numerous ova of *F. magna*. The negative results of the fecal examinations cannot be explained. One large mature trematode was recovered from a cavity under the aforementioned projection, and a small immature specimen was found in the liver tissue beneath the abomasal impression. A gross section of the liver revealed extensive damage to the tissue of the right lobe brought about by the migration of the immature parasite (Plate II, Fig. 1). The cut surface of the lobe revealed 32 cross sections of a migration route, which was in the form of a convoluted channel at the end of which the trematode was found.

The calf, which had been experimentally exposed to infestation over a period of 141 days, having ingested a total of 180 metacercariae (cercariae ex *Fossaria parva*), was slaughtered and autopsied on the 261st day following the first exposure, which was also the 120th day after ingestion of the last lot of metacercariae. This animal had remained in fair condition throughout the experiment, although gain in weight may have been somewhat retarded. Differential leucocyte counts were made from the peripheral blood on 12 successive days prior to slaughter. These counts are given in Table I.

The high eosinophila shown by these counts is remarkable. This feature, combined with high lymphocyte, low neutrophile, and normal monocyte counts is typical of the picture seen in the healing phase of a septicaemia, and appears to indicate repair of tissue damage.

The autopsy revealed the liver to be heavily infested with *F. magna*. The surface of the organ was irregular and many areas showed the black pigmentation as described by other workers from livers of *Bos taurus* naturally infested with the parasite. The lymph glands of the liver were black in color

TABLE I

—	Neutrophiles, %	Lymphocytes, %	Monocytes, %	Eosinophiles, %	Basophiles, %
1	23.5	38.0	12.5	25.5	0.5
2	16.0	52.5	8.0	23.0	0.5
3	11.0	47.5	10.0	31.0	0.5
4	11.5	60.5	13.5	13.0	1.5
5	20.0	47.5	11.0	21.5	0
6	18.0	47.0	10.5	24.5	0
7	9.5	52.0	9.5	28.5	1.0
8	17.5	47.5	10.0	25.0	0
9	15.0	50.0	7.0	27.0	1.0
10	15.5	53.0	11.0	20.5	0
11	13.0	47.5	9.0	29.0	1.5
12	11.0	52.0	6.5	30.5	0
Average	15.1	49.55	9.85	25.27	0.46

and the omentum was spotted with the pigment (Plate I, Fig. 4). The gall bladder and hepatic duct did not contain any ova of *F. magna.* A single cross section through the greater plane of the organ disclosed ten immature trematodes. Subsequent examination revealed the presence of several more specimens, two of which were mature. Owing to the fact that a large part of this liver was retained as a museum specimen, the total number of trematodes present was undetermined. This experimental animal thus added strength to the hypothesis that *Bos taurus* cannot act as a normal definitive host for *F. magna*, and provided tissue specimens for histopathological studies.

Microscopical examination of the feces of eight cattle (*B. taurus*), which ranged over an area from which several other animals had been found on autopsy to be infested, did not reveal the presence of trematode ova. In this enclosure one of the intermediate hosts was present in large numbers and had been exposed to the feces of infested Cervidae. The above-mentioned findings merely add weight to the hypothesis that Bovidae are not normal definitive hosts.

It can be stated quite definitely at this time that there are three distinct defence reactions to invasion by *F. magna*, as seen in the large Bovidae, the Cervidae, and in *O. aries*. These reactions are degrees of vital resistance to the invasion, considered from the point of view of the parasite. The large Bovidae are completely resistant, the Cervidae are tolerant and *O. aries* is partially resistant to *F. magna*, these definitions being made with the understanding that the phenomena are considered as degrees of resistance in so far as the normal life of the parasite is concerned, and that they do not refer to the host's immunity to fascioloidiasis magna.

It was seen that a celluar basis existed for each degree of resistance, and that these bases differed from one another in a quantitative and also, to a lesser extent, in a qualitative way. A study of the structure of the "defence reactions" was made in order to record the active cells in each basis.

The Cellular Bases of the Defence Reactions

The following observations are based upon studies of the preparations from lesions in *Cervus canadensis*, *Odocoileus virginianus*, *Bos taurus*, *Bison bison*, *B. taurus* × *B. bison* and *Ovis aries*. The tissues were fixed in formalin and the blocks were prepared by standard methods, butyl alcohol being used as the intermediary between alcohol and wax. Sections were cut from 8-10μ in thickness, depending on the tissue. Two staining methods were used on each specimen, eosin-haematoxylin for the study of cellular infiltrations and Van Gieson's picric-acid fuchsin in conjunction with Harris' aqueous haematoxylin for demonstrating fibrous tissue structures.

The lesions in *C. canadensis* were collected from two aged males, a yearling male, and a three-year-old male, which were parasitized by 14, 40, 15 and 18 trematodes respectively. This material included cavities containing mature trematodes, a young trematode migrating through the tissue, a young trematode becoming encapsulated, the walls of a closed cyst, the path of migration of a young trematode, a fibrous cavity containing an immature specimen, an enlarged bile duct containing one trematode, and portions of liver from between a lesion and the hilum hepatis.

The tissue from *O. virginianus* represented the cyst wall and surrounding parts of lesions containing large mature trematodes.

In addition to the tissue of *B. bison* previously studied (10), tissues from four more of these animals were prepared. These preparations represented lesions containing mature trematodes and showing surrounding liver lobules, tissue containing small immature trematodes partly encapsulated, pigmented tissue and portions of liver from around the lesions.

Tissues from two livers of naturally infested *B. taurus* × *B. bison* were prepared; these were prepared as 15 sections through lesions containing mature and dead trematodes, and sections of surrounding liver tissue. From the liver of the experimentally infested *B. taurus*, 11 preparations of lesions and surrounding tissue were obtained. These were prepared for comparison between this animal and the naturally infested Bovidae, and to show lesions caused by immature parasites.

The livers of three experimentally infested sheep (*O. aries*) were studied. Twenty-nine sections were prepared from the channels of migration, the migrating trematodes, the walls of a large cavity which contained a mature parasite, liver tissue surrounding lesions, and tissue some distance from microscopic lesions.

Cervidae

1. *Complete Cavities in C. canadensis*

The wall of the cavity is composed of two layers—a richly cellular inner layer of fibrous tissue and a comparatively acellular outer layer of denser fibrils. These layers are separated by a fairly well defined line of demarcation, made by a sudden decrease in the number of cells present in the tissue.

The cellular layer in the specimens examined is from 0.094 to 0.312 mm. and the outer layer from 0.625 to 1.4 mm. in thickness; these figures represent the measurements of what the writer considers to be typical specimens, although it is probable that all lesions in *C. canadensis* will not fall within the above ranges. The cellular layer is composed of fine collagen fibrils and fibroblasts, both phagocytic and non-phagocytic mononuclear exudate cells, eosinophiles, and a very few polymorphonuclear leucocytes. From counts of two areas on each of five preparations of the cellular layers from different lesions the following percentages of the cells were estimated to the nearest 1%:

Polyblasts	68 to 80%; average 74%
Fibroblasts	16 to 24%; average 21%
Eosinophiles	1 to 7%; average 4%
Polymorphonuclear leucocytes	0 to 1%; average 1%

The above counts were made from lesions which were judged to be typical for this host; about one thousand cells were counted in each area. Adherent to the inner surface of the wall are a few erythrocytes and particles of pigmented detritus.

The more fibrous layer of the wall is composed chiefly of collagen fibrils which gradually increase in density towards the outer edge of the wall. Fibroblasts are interspersed in the fibrils but become less numerous towards the outer edge; the organization of fibrous tissue becomes more complete towards the outer edge, but completely acellular tissue has not been seen in this host.

As previously reported, many of the small bile ducts, intercepted in their course by the formation of the capsule, have not been occluded by the organization of the wall and as many as 16 are seen in a single cross section, some containing ova and detritus. The wall has a blood-vascular system; in most preparations the number of cross sections of vessels was found to vary between 200 and 300, including small capillaries.

The columnar epithelium of the bile ducts is similar in appearance to that of the main ducts in fascioliasis hepatica. The epithelial cells have proliferated, forming assemblages of cells which project into the surrounding tissue in forms similar to foreign body giant cells. Although this formation may be seen in large normal ducts it is not present in normal ducts of similar size to those in a lesion of fascioloidiasis. At the point where a duct enters or leaves the cavity these cells may be seen on the inner surface of the wall adjoining the opening.

The lobules in immediate contact with the fibrous wall are composed of normal liver cells but scattered throughout the chains are eosinophiles, which number 50-100 in each lobule. In an average section several portal canals show the presence of an abnormal amount of fibrous connective tissue. In these inter-lobular canals, the bile duct is sometimes occluded and particles of pigmented detritus are present. These particles compose the black spots seen macroscopically in the tissue of an infested liver. The interlobular ducts, owing to their small size, are unable to convey ova of *F. magna*, but they apparently attempt to carry detritus from the lesion; this detritus causes a low-grade inflammatory process leading to the formation of fibrous tissue and occlusion of the canal, as would any foreign body.

2. *The Tissue Surrounding a Migrating Trematode in C. canadensis*

The cellular reaction of *Cervus canadensis* to the migrating trematode has been studied in specimens in which the parasite is from 1.3–1.7 cm. in length. The tissue in contact with the trematode is similar in general appearance to the cellular layer of the wall of a well formed cavity, but numerous erythrocytes and particles of pigmented detritus are intermingled in the tissue. The portion of the lesion in contact with the anterior parts of the migrating parasite is in the form of a very thin layer of protective cells; these cells are fibroblasts with fine collagen fibrils, eosinophiles, mononuclear exudate cells (phagocytic and non-phagocytic) and polymorphonuclear leucocytes. The fibroblasts are numerous and the polymorphonuclear leucocytes are very few in number. The eosinophiles are more numerous than in established lesions, and tend to congregate in clumps near the normal liver cells. Towards the posterior part of the parasite the protective layer becomes thicker, but the only noticeable change in its cellular constitution is a proportionate increase in the number of fibroblasts. In the channel behind the parasite there is a mass of erythrocytes, pigment and plasma. The liver tissue immediately surrounding the migration channels is damaged; this damage is seen as displacement of liver cells in an area of two lobules on each side; the cells have been forced out of their typical radiating chain formation, the chains being parallel to the channel and the cells slightly atrophied. The nuclei of the cells appear to be normal and it is probable that complete regeneration of the lobules follows the cessation of the pressure of infiltration and organization of fibrous tissue.

The cellular elements were counted in several fields of the tissue showing defence reaction around the parasite and the cellular layer was found to be constituted approximately as follows:

Polyblasts	70–72%
Fibroblasts	16–20%
Eosinophiles	8–13%
Polymorphonuclear leucocytes	0– 1%

Owing to the difficulty in seeing the poorly staining cells in the masses of detritus particles, the above-mentioned percentages are not as reliable as those counted in the cellular layers of established and clear lesions.

3. *Complete Cavities in O. virginianus*

The wall of the capsule is essentially the same as it is in the complete cavities in *C. canadensis*. In the available specimens the outer layer is less cellular and the fibres of connective tissue are more dense. The cellular inner layer lacks the eosinophiles noted in the former host, this possibly being due to the age of the lesion. In other respects the proportions of cellular elements are very similar.

The wall includes a similar blood-vascular system and the bile ducts intercepted by the cavity are open and are active in removing detritus and ova.

The lobules immediately surrounding the lesion are normal and do not show specks of pigmentation. Thus the portal canals are normal and there is no evidence of a continued tissue reaction to the egress of waste products by way of the ducts. On the whole, *O. virginianus* appears to be at least as tolerant to *F. magna* as is *C. canadensis*.

LARGE BOVIDAE

1. *Lesions Containing Mature Parasites in Bison bison*

All these lesions have been found to be complete cysts with thick walls of dense fibrous tissue. The walls are very irregular in outline and include masses of detritus and ova (Plate II, Fig. 2). The outer fibrous layer projects into the surrounding liver tissue and in some cases isolates islands of liver cells. The masses of ova and detritus are always surrounded by a cellular layer of tissue which is composed of very variable proportions of polyblasts (chiefly phagocytic mononuclear exudate cells) and fibroblasts. These layers have not been found to include polymorphonuclear leucocytes.

The wall of the true cyst is not so clearly defined as is the wall of the cavity in Cervidae, owing to the above inclusions. However, it is distinctly divided into a cellular and a comparatively acellular layer. Both layers are difficult to describe owing to the extremely variable thickness of the cellular layer and the numerous inclusions and consequent irregularities in the fibrous layer. All bile ducts in and approximate to the lesion are completely occluded by extensive fibrosis; the sites of former ducts are marked by prolonged areas of fibrosis surrounding detritus. A blood-vascular system is present in the fibrous layer of the wall, the vessels being present chiefly in the part approximating the liver tissue.

Fibrous connective tissue is continually being formed on the inner surfaces of the cavity; this fact is shown by the large numbers of ova in the wall which are seen in increasing numbers towards the inner surface but are more firmly encapsulated towards the outer surface. In one lesion, which was sectioned in several places, more than 4,000 ova were present in the wall in each section of ten microns; this indicated the great number of ova present in the wall which enclosed only two trematodes. This fact shows that encroachment of fibrous tissue had been in progress for a considerable time even after the parasites had been completely encapsulated and all bile ducts occluded. This continued fibrous tissue response to the presence of the parasite constitutes the most spectacular difference between the defence reactions of large Bovidae and those of the Cervidae to *F. magna* and its products. The ova in the deeper layers of the wall are more free from detritus owing to phagocytosis having taken place.

The elements in the clearer areas of the cellular layer in the lesions in *B. bison* were found to be in the following approximate proportions:

Polyblasts	75%
Fibroblasts	24%
Eosinophiles	1%
Polymorphonuclear leucocytes	0

In typical lesions there are isolated groups of cells which are purely eosinophiles. These groups have not been found in the inner cellular layer but are always approximating areas in which there are numerous small particles of detritus. This detritus, which is undoubtedly the black vomitus of the trematodes, would attract only phagocytic cells and it appears that the function of the eosinophiles in this position is purely phagocytosis.

A section through an entire lesion containing mature trematodes in *B. bison* shows a conglomeration of local cellular reactions amid irregular masses of dense fibrous connective tissue. The space in the actual cavity not taken up by the bodies of the trematodes is filled with a mass of vomitus, polyblasts and a few eosinophiles.

The liver tissue surrounding the lesion is normal in appearance, although in some islands that are surrounded by fibrous tissue the radiating chain-like formation of the cells is disrupted, even although the cells themselves appear normal.

The occluded ducts leading from the lesion towards the hilum hepatis are marked by lines of fibrosis. They are completely occluded for some distance after leaving the lesion and in cross section their appearance is essentially the same as the one shown in Plate III, Fig. 5.

Lesions caused by migrating trematodes or those containing sexually immature specimens have not been found in *B. bison.* Large egg-like masses of dense fibrous tissue are common. These masses could not be readily sectioned; they were surrounded by normal liver tissue and did not contain particles of pigmented detritus.

2. *Lesions Containing Mature Parasites in B. bison × B. taurus*

Large fibrosed lesions, similar to those collected from *B. bison*, were the only ones available in the material from several specimens of this hybrid host. The parasites were likewise completely enclosed in fibrous cysts, the walls again appearing as irregular conglomerations of fibrous tissue and local cellular reactions to the presence of detritus. Several sections of the liver tissue were obtained from between the lesion and the hilum hepatis which showed the complete occlusion of the lumina of bile ducts. In no case has an open duct been found in the lesions from this host. The cysts were filled with detritus and ova and it is of interest to note that the sections of the intestinal caeca of the parasites showed the presence of numerous ova; this showed that the enclosed material in the cyst was ingested in place of normal food.

The walls of the cyst, which measured from 1.0 to 2.3 mm. in total thickness, showed the presence of the cellular and fibrous layers, but as in the case of *B. bison*, they were irregular. The cellular elements showed no differences from those in *B. bison.*

3. *Lesions in Bos taurus*

In this host it was possible to obtain the important lesions caused by the migrating parasites in large Bovidae. The microscopical appearance of the tissue surrounding a migrating specimen is strikingly different from that of a similar lesion in *Cervus canadensis.*

The channel of migration directly behind the parasite is filled with red blood corpuscles and detritus as in the other hosts, but this mass is surrounded by a dense layer of eosinophiles which is from 0.8 mm. to 1.3 mm. in thickness. These cells are seen to be actively phagocytosing particles of the pigmented detritus (Plate III, Fig. 6). As many as 15 particles are seen in the cytoplasm of many of the eosinophiles; other cells can be seen with particles adhering to their surfaces as if the particles had been opsonized. In some cases the eosinophiles appear to be phagocytosing the erythrocytes. This active phagocytosis of inert particles and cells by eosinophiles is in conformity with the observations of Weinberg and Séguin (14, 15). As in sections from *B. bison* the eosinophiles are definitely more numerous around the inert particles than around the parasite and this has led me to believe that the particles of vomitus are strongly eosinotactic, and that phagocytosis is at least the primary function of these cells in fascioloidiasis magna of large Bovidae.

The eosinophile layer is in turn covered by another cellular layer in which numerous fibroblasts and polyblasts are seen in a dense network of fine collagen fibrils. In smaller numbers are eosinophiles, degenerate liver cells and inert particles. In this layer the phagocytic mononuclear exudate cells are active in removing inert particles of detritus.

The liver cells surrounding the above layer are normal in individual appearance, but in some places the cell chains are disorganized. In all lobules within a radius of 1.5–1.75 cm. from the channel fine collagen fibrils are intertwined in the cell chains, and the number of fibroblasts present in the lobules is only slightly less than the number of liver cells. The lumen of the central vein in some lobules is filled with eosinophiles and non-phagocytic polyblasts. The portal canals are involved in the reaction, the connective tissue showing hypertrophy and cellular infiltration.

Sections showing the parasite in the process of becoming encapsulated are essentially the same as above in general appearance. The amount of fibrous tissue is somewhat increased; the collagen fibrils are more numerous and are stronger. Fewer eosinophiles are present, but the phagocytic mononuclear exudate cells appear to be present in similar numbers.

Ovis aries

1. *A Lesion Containing a Mature Parasite*

The wall of the cavity is a thin layer of delicate fibrils and fibroblasts; it varies in thickness from 0.4 to 0.94 mm. The fibrosis involves the surrounding liver tissue to some extent by irregular projections of fibrous tissue. The wall is not divided into cellular and comparatively acellular layers; numerous polyblasts and a few polymorphonuclear leucocytes are evenly intermingled with the fibroblasts and fibrils throughout the wall. The weakness of some parts of the wall of the cavity suggests that the trematode might have been able to break through and resume its migration had conditions become unfavorable for its existence in this location.

The route taken by the parasite to the point of encapsulation is seen as a wide channel in which the destroyed tissue has been replaced by loose fibrous tissue. Phagocytosis of the detritus has been completed and nearly all of the cells present in the channel are fibroblasts. For a short distance along the channel is a line of loosely encapsulated ova which had evidently been produced by the trematode at the time it was migrating. The presence of these ova in the channel indicates that the parasite migrated for a period of somewhat more than five months before becoming encapsulated and as the parasite was 206 days old at the time of death it had evidently been in the final lesion for somewhat less than 56 days.

The egress of the ova from the cavity was by way of a small bile duct which leads from a point near the end of the migration channel towards the hilum hepatis. Ova and detritus are present in the lumen of the duct; the epithelial layer is somewhat hypertrophied, and many polyblasts (phagocytic mononuclear exudate cells) are present in its folds.

2. *The Channels Made by Migrating Trematodes in O. aries*

The channels have lumina of varying diameters which are filled with pigmented particles of vomitus, erythrocytes and disintegrating liver cells. Surrounding and intermingled with this material are irregular masses of polyblasts and polymorphonuclear leucocytes, and a few eosinophiles; the cellular infiltration is, however, very sparse when compared with similar lesions in *B. taurus*.

The eosinophiles appear to be phagocytosing small inert particles and the monocytoid polyblasts are removing larger particles. The exudate cells extend into the sides of the channel, only changing their character in the total number of cells present. Outside of this layer the fibroblasts appear with accompanying collagen fibrils; these fibrils are very delicate in comparison with those in the Bovidae and do not appear to have the same power of reparation (Plate II, Fig. 5). On the outer edges of this lesion a few fibrils are seen intertwined in the cell chains of surrounding lobules.

The liver tissue immediately surrounding the migrating parasite is somewhat disorganized; the cell chains in adjacent lobules have been forced out of normal position and lie parallel to the channel. Between the anterior end of the trematode and the normal liver tissue is a thin layer of detritus similar to that in the channel behind, but there is no evidence of fibrosis as seen in the other hosts. In no case have two parasites been seen migrating together in a single channel.

In the whole of the liver tissue in each of the three sheep studied a severe fatty infiltration has been observed. Even the peripheral portions away from an actual lesion have shown this condition as severely as the tissue seen in Plate III, Fig. 2. Fatty degeneration has not been seen; the nuclei in the affected cells are normal in structure but displaced in position by the fat globules. Tissue taken from the antero-ventral border of one liver showed infiltration with polymorphonuclear leucocytes and polyblasts, as well as the fatty infiltration.

Discussion of the Tissue Defences in Fascioloidiasis Magna

Fascioloides magna migrates through the liver tissue of its definitive host causing destruction of the lobules. Most of this destruction ceases when the tissue reaction of the host causes encapsulation of the parasite by fibrous connective tissue. Thus, the manifestations of fascioloidiasis magna in various ruminants are governed by the following host responses:

(1) The fibrous tissue reaction to the presence and activity of the parasite.
(2) The ability of the phagocytic cells to remove detritus.
(3) The regenerative power of surrounding liver tissue.

It is seen that the defences of different hosts may differ considerably in detail, even though the encapsulation of the parasite is certainly the objective in all cases. The details of defence have been found to be more or less constant within certain groups of ruminants but they differ between these groups. These details have a profound effect upon the life of the parasite as well as being all important in regard to the manifestation of clinical disease.

The excessive formation of fibrous tissue in the Bovidae successfully prevents undue damage to the liver of the host as well as precluding continuance of the life cycle. This power of encapsulating the parasite and its products appears to be accompanied by an exudation of great numbers of eosinophiles, and the collagen fibrils formed are undoubtedly stronger than those in the other host groups. This defence activity is not confined to *F. magna* but is seen in the "pipe-stem" liver condition in fascioliasis hepatica in *Bos taurus*, in the fibrous nodules formed around the remains of the filarial nematode, *Setaria labiato-papillosa;* it is also seen, as noted by Dévé (2) in echinococcosis granulosa. It may also be found that *Onchocerca* spp. are so firmly fibrosed that reproduction of the nematode is checked in these animals.

In fascioloidiasis magna the fibrous tissue reaction in Bovidae appears to be continued, even after the parasite is completely encysted, until the whole lesion is fibrosed and phagocytosis is complete. In arriving at this end the process throughout has been one of chronic inflammation localized around the parasite, its products and migration channel, and also local cirrhosis.

The entirely different pathological processes in *Ovis aries* are characterized by a continued acute inflammatory reaction around the damaged tissues, as judged by the presence of comparatively numerous polymorphonuclear leucocytes and the profound general effect on the liver tissue. The inability of this host to check the migration, and hence continued damage, appears to be due to the weakness of the collagen fibrils, which, in turn, appears to be a characteristic of the host species. The absence of a marked eosinophilia in the specimens observed may possibly be concerned in the unchecked migration of the trematode, or it may be that these leucocytes function only in parasitic diseases when the inflammatory reaction is chronic or productive in type.

The Cervidae appear to be entirely suitable for the role of definitive hosts of *F. magna*, the host reaction to the effects and presence of the parasite being adequate to prevent undue tissue damage but not severe enough to prevent reproduction by completely encysting the organism. This tolerance to the effects of a helminth is observed in other helminthiases, filariases for example, and appears to be a natural characteristic of Cervidae rather than a specific tolerance to *F. magna*.

From the immunological point of view it is suggested that the large Bovidae be considered as naturally resistant to the presence of *F. magna*, to the extent of preventing reproduction. *Ovis aries* is a compatible host, but is also intolerant from the parasite's point of view, in that the result of the parasite's invasion is clinical disease which may preclude reproduction. Cervidae are compatible hosts and are tolerant to the effects of the parasite.

In these studies it was unfortunate that the material was limited insofar as tissue from *Bos taurus* is concerned. However, it is hoped that further work of this nature will be done from the point of view of comparative pathology in the case of other helminthiases caused by parasites that invade parenchymatous tissues.

A great number of animals would have to be studied in order to prove beyond doubt that large Bovidae are unable to act as normal definitive hosts in the life cycle, but data now on hand make it reasonably certain that these animals will not spread the parasite nor will they become infested unless they graze on land occupied by infested Cervidae or *Ovis aries* or both. Sheep should be prevented from grazing on snail infested land occupied by Cervidae, particularly as *O. aries* is the only host observed in which *F. magna* produces severe clinical disease.

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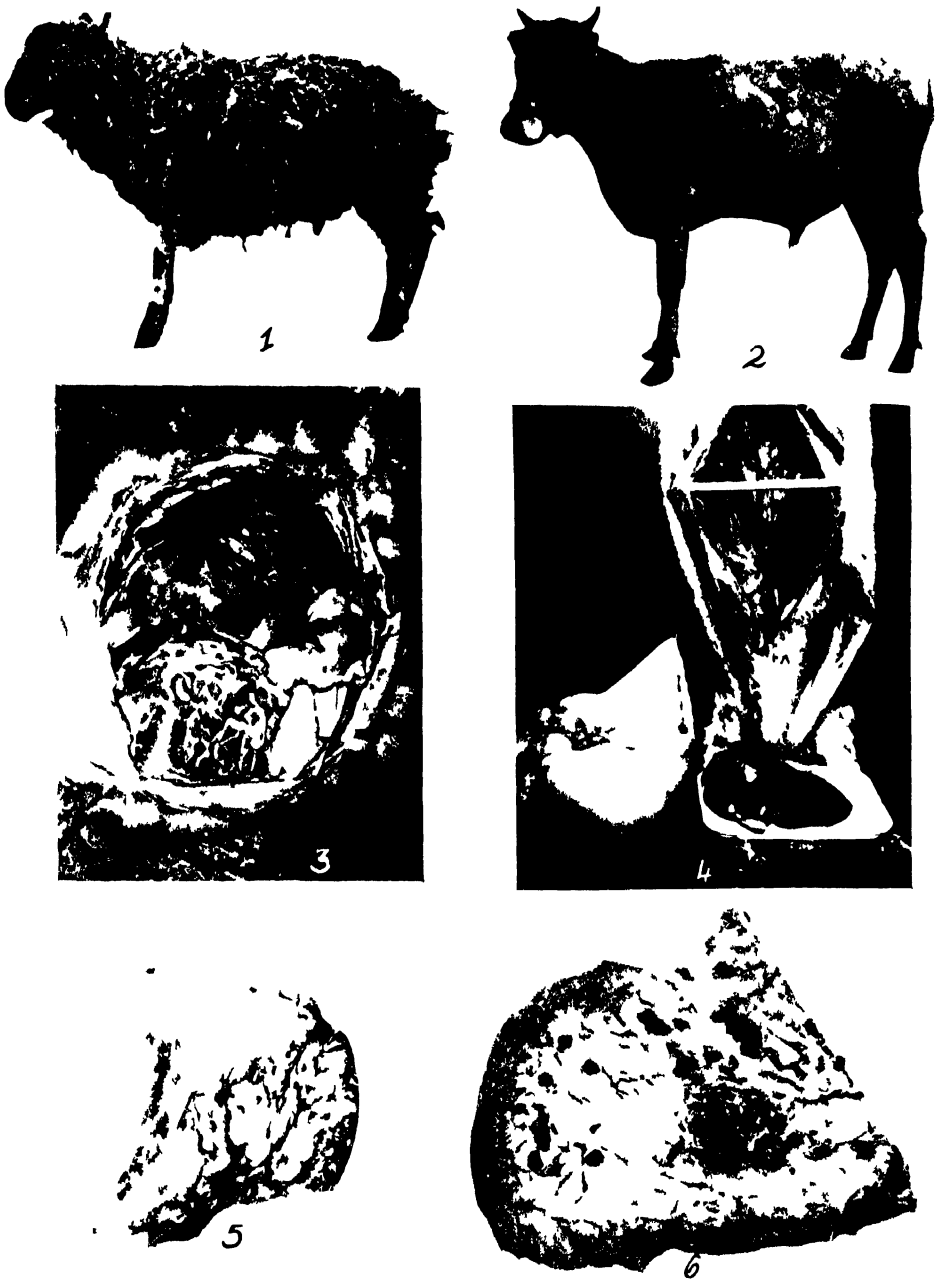
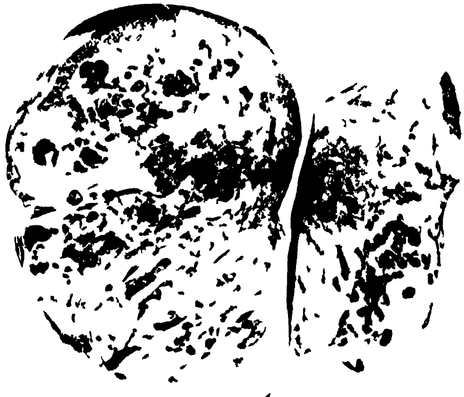


FIG. 1. *Sheep No. 3 on the 204th day of infestation with two trematodes.* 2. *A young bull on the 261st day of infestation with numerous trematodes.* 3. *The appearance of the liver in situ of Sheep No. 3 on the 206th day of infestation.* 4. *The autopsy of the above bull.* 5. *The liver of Sheep No. 2 parasitized by seven trematodes.* 6. *A gross section of a lobe of the liver of the above B. taurus showing the migration channels of nine trematodes.*



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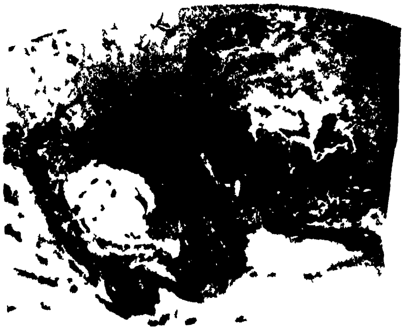

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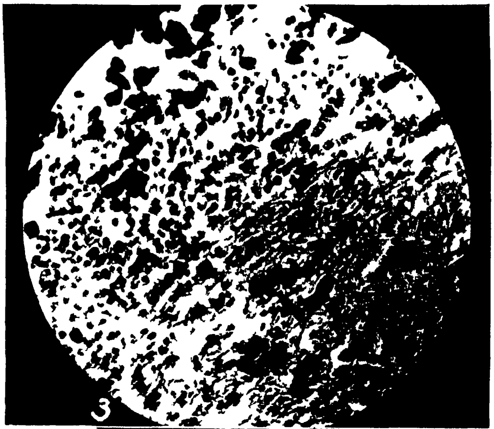

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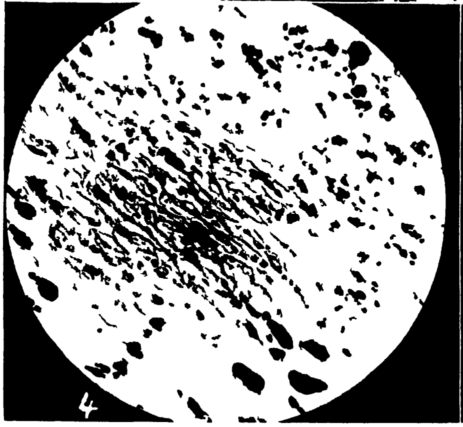

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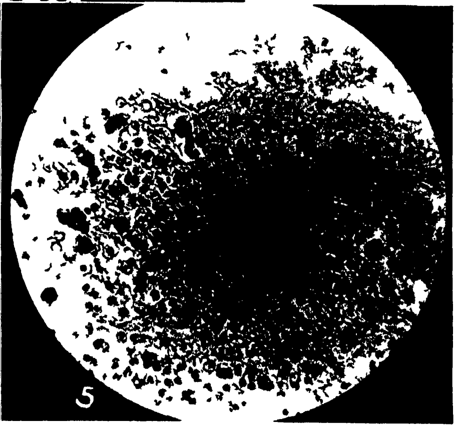

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FIG. 1. *Gross sections of the livers of Sheep Nos. 2 and 3. The liver on the left parasitized by seven immature parasites. The portion on the right shows the migration channel of one immature specimen.* 2. *A section of a typical lesion in B. bison containing two mature trematodes and showing the excessive fibrosis (× 1.6).* 3–5. *The fibrous tissue reaction around typical migration channels in B. taurus, C. canadensis and O. aries respectively.*

PLATE III

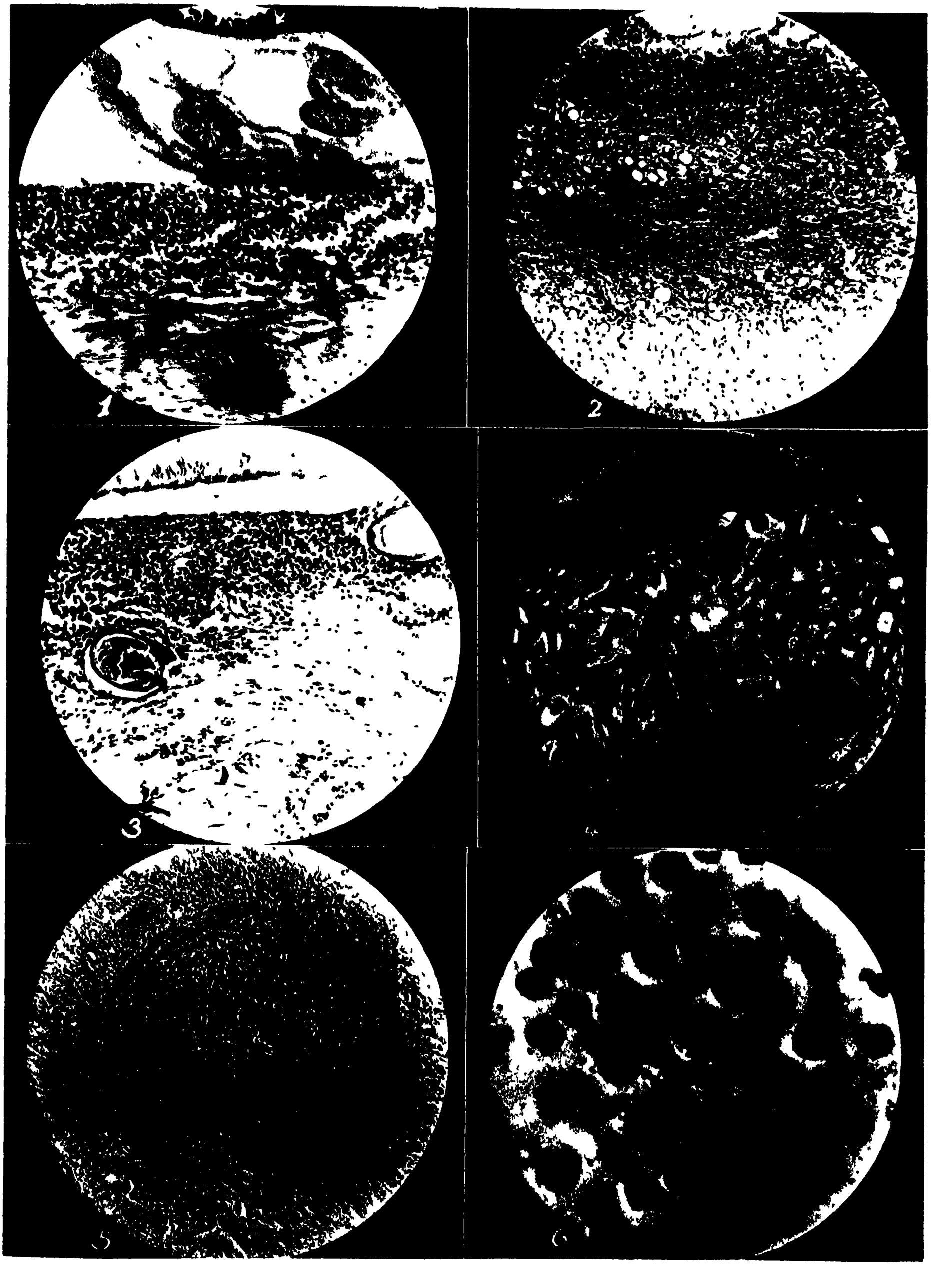

FIG. 1. *A section of a lesion containing a mature parasite in C. canadensis, showing a part of the cavity, the cellular layer, and part of the fibrous layer of the wall* (× 97). 2. *A section through the entire wall of a lesion containing a mature parasite in O. aries* (× 83). 3. *A section of a lesion in B. bison showing the features of Fig. 1 for comparison* (× 97). 4. *The wall of an enlarged hepatic duct which contained a mature parasite in C. canadensis* (× 40). 5. *The occlusion of a bile duct adjacent to a lesion in B. taurus × B. bison* (× 84). 6. *Eosinophiles in a migration channel in B. taurus in the act of phagocytosing granules of detritus.*

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THE BLACKFLIES OF EASTERN CANADA (SIMULIIDAE, DIPTERA).[1] PART I.

BY C. R. TWINN[2]

Abstract

Twenty-three species and one variety belonging to three subgenera of the family Simuliidae are recorded and described. Of these, twelve species and the variety are new to science. Only two of the remaining eleven species of other authors have been previously recorded in Eastern Canada; two others, originally described in Europe, are recorded in North America for the first time, and the hitherto unknown male of a fifth species is described. The adult forms and the pupae and cocoons of these species are described, and the genitalia of both sexes of flies are figured. In addition, notes on the habitats, distribution, species associations, and similar information of value in identifying the species are given. Keys to the subgenera and species, including both sexes of adults and the pupae are an important feature.

Introduction

Blackflies have long been recognized as among the worst bloodsucking insect pests affecting wild and domestic animals and man, in many parts of the world, and the literature contains numerous references to their serious depredations. Among the most famous and spectacular are the outbreaks of the buffalo or turkey gnat, *Simulium pecuarum* Riley, in the lower Mississippi valley of the United States, and of the Golubatz fly, *S. columbaczense* Schoenbauer, in parts of south-eastern Europe. The latter species was reported to have caused the deaths of many thousands of wild and domestic animals in Rumania in 1923. In recent years, blackflies have been implicated in the transmission of disease-producing organisms, and it is probable that further studies will prove them to be of greater importance in this connection than is at present known. In 1926, Blacklock (1) discovered that the intermediate host of *Onchocerca volvulus* Leuckart, a filarial worm causing disease in man in West Africa and Uganda, is the blackfly, *Simulium damnosum* Theo. Another filarial worm, *O. caecutiens* Brumpt, possibly only a variety of *O. volvulus*, which infests man in Mexico and Central America, was shown by Hoffman (22), in 1930, to pass its intermediate stages in the blackfly, *S. mooseri* Dampf; since then certain other species of blackflies have also been proved to be vectors of the organism. O'Roke (34)demonstrated that a serious protozoon blood parasite of ducks, *Leucocytozoon anatis* Wickware, prevalent

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Contribution from the Entomological Branch, Department of Agriculture, Ottawa. This paper was presented at McGill University, Montreal, in partial fulfilment of the requirements for the degree of Master of Science.

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in North America, passes the sexual stage of its life cycle in the blackfly, *S. venustum* Say, transmission in nature being effected by the bite of the fly. Both this fly and the disease are widespread in Canada, as I have reported elsewhere (55).

The published information on the blackflies of Canada, especially of Eastern Canada, is very meagre. The late Eric Hearle, in 1932, prepared (20) a useful annotated list of the blackflies of British Columbia in which he included twenty species, two of which were new. Twinn (55), in 1933, published a short article on the general biology and distribution of *Simulium venustum* Say, in Canada. In 1927, Dyar and Shannon (5) published a revision of the Simuliidae of North America. Only two of the 47 species included were shown as occurring in Eastern Canada.

Scope of Work

The present paper includes twenty-three species and one variety belonging to three subgenera of the family Simuliidae. Twelve of these species and the variety are described as new to science. Of the remaining eleven species of other authors, only two, *venustum* Say and *johannseni* (Hart), have been recorded previously in Eastern Canada; two, *subexcisum* Edw., and *latipes* Mgn., originally described in Europe, are recorded in North America for the first time; and the hitherto unknown male of *mutatum* (Mall.) is described, and the pupa associated with the adult forms.

The treatment followed throughout is essentially taxonomic. The adult forms are described, and brief descriptions of the pupae and cocoons are given; also notes on the habitats, distribution, species associations, and similar information of value in segregating and determining the species. Keys to the subgenera and species are presented; the latter include both sexes of adults and the pupae and cocoons, and should prove to be a valuable feature.

It is hoped that an opportunity will later arise to extend the study to include adequate treatment of the immature stages, and the biology of the various species. Much material and information have already been brought together toward this end.

Material and Methods of Study

The study is based on material collected in the territory surrounding Ottawa, in Ontario and Quebec, known as the Ottawa district, and on specimens from other parts of Eastern Canada, including Baffin Island. However, the number of specimens in the National Collection, or received from collectors in this region was small, and therefore the work has been carried out largely with material collected and reared by me in the Ottawa district. This work was done with the permission of the Dominion Entomologist. Numerous collections of immature stages were made prior to 1935, but the systematic examination of breeding places, and rearing of adults from pupae was done, for the most part, during the spring and summer of 1935.

In the course of the investigation (necessarily restricted by other duties), five rivers including the Ottawa, Rideau, Blanche, Jock and Mississippi, and sixteen permanent, semi-permanent and temporary streams and rills, all within a few miles of Ottawa, were visited and examined at definite points, at intervals from late April to the end of September. During these visits notes were taken on the seasonal character of the water bodies, their rate of flow, temperature, and other data; more than 300 alcohol collections were made of the blackfly stages found, and live pupae were placed on moist cotton in stoppered vials for rearing, all being associated by means of experimental numbers.

The adult flies were reared from the pupae, after being roughly segregated by species under the low power objective of the microscope, by means of the number, arrangement and character of the filaments comprising the respiratory organs, and the nature of the cocoons. When *Simulium* pupae are kept moist (not wet) they will complete their development even though removed from their natural habitat, and will give rise to imagines, which may be transferred to dry vials by taking advantage of the positive phototropic responses of the flies.

Rearing the adult flies in this manner is undoubtedly the most satisfactory method of securing material for a taxonomic study of the Simuliidae. By this method both sexes are secured in excellent condition, whereas if one confines his activities to collecting with a net he usually secures only the females, and these often in poor condition. Moreover, the rearing method definitely connects the adults with their immature stages, concerning which there has been much confusion, and establishes the nature and location of the breeding places. When the pupae are known, it is comparatively simple to identify the larvae, this being accomplished merely by dissecting out and examining the filaments in the pupal respiratory histoblasts, in the prothorax of the mature larva.

A satisfactory method of rearing adults from pupae consists of using glass vials 1¼ × 6 in., placed on their sides; in these, a narrow strip of cotton batting, wet, but with the surplus water removed, is inserted lengthwise along the glass, and the pupae gently placed on it. A similar vial containing a strip of dry blotting-paper is placed with its mouth against that of the vial containing the pupae. The two vials are then wrapped in paper in this position, the paper held in place by rubber bands and completely covering the vial containing the pupae, but leaving two or three inches of the emergence vial uncovered. When the flies emerge they are attracted by the light into the emergence tube, where the blotting paper absorbs any surplus moisture and provides them with a resting place. The flies were usually left for 18–24 hours after emergence to allow the body integument to harden before pinning. The pupal skins and cocoons were preserved in alcohol-glycerine solution under the same experimental number as the pinned adults; when the latter were numerous some of them were also preserved with the skins.

The Characters of the Group

Blackflies are small, compact, stoutly-built flies, with the thorax greatly developed and curiously arched. The wings are broad with the anterior veins thickened, and the others indistinct; the legs are short and strong; the abdomen is nine-segmented. The antennae are short and typically eleven-jointed, but sometimes ten or nine-jointed; the palpi are four-jointed. The eyes are large and holoptic in the male, dichoptic in the female, the upper facets of the male much larger than the lower*; ocelli are absent. The mouthparts of both sexes are adapted for piercing, but only the females suck blood. The immature stages develop in running water. Illustrations of the eggs will be found in Plate I, Figs. 1, 2; of the larvae, in Plate I, Fig. 3; of the pupae, in Plate I, Figs. 4, 5, 6. Puri (41) has published an excellent account of the life history and structure of the early stages of Simuliidae; Johannsen (23, 24), Pomeroy (38), Edwards (7) and others, have also made important contributions to this interesting subject.

Explanatory and illustrated accounts of the wing venation and other external characters of the Simuliidae, a knowledge of which is necessary for students of the group, will be found in the works of Malloch (29), Edwards (6 and 8), Pomeroy (38) and Dyar and Shannon (5), and need not be repeated here. Two terms used in the following keys and descriptions to describe certain important tarsal characters were adopted by Edwards (8, p. 122) from Enderlein. To prevent possible confusion, Edwards' definition of these terms is given as follows: "(1) *Pedisulcus*, the dorsal incision near the base of the second segment of the hind tarsi; and (2) *Calcipala*, the flattened projection at the tip of the first segment (basitarsus) of the hind tarsi, projecting beyond the base of the second segment on the outer side".

In the taxonomic study of the material brought together as already outlined, the genitalia of both sexes were studied, in addition to external characters and the characters of the pupae and cocoons. This work entailed a great deal of delicate and painstaking dissection work under the binocular microscope, and the making of more than 300 permanent microscopic preparations mounted in euparal on slides. Furthermore, 93 separate drawings illustrating the genital structures were made with the aid of a camera lucida, and are included in this paper. These were all drawn to the same scale and reveal quite striking variations in the size of the genitalia of the different species, as well as in form.

The use of the male genitalia in taxonomic studies of the Simuliidae is comparatively recent. According to Edwards (6, p. 23) and Gibbins (17, p. 317) Lundstrom (27), in 1911, was the first to pay attention to these structures, in an account of the Finnish species, but he did not study them closely and his drawings were not sufficiently detailed. Edwards (6) made use of them in 1915, in describing the British species of *Simulium*, and also in a paper (8)

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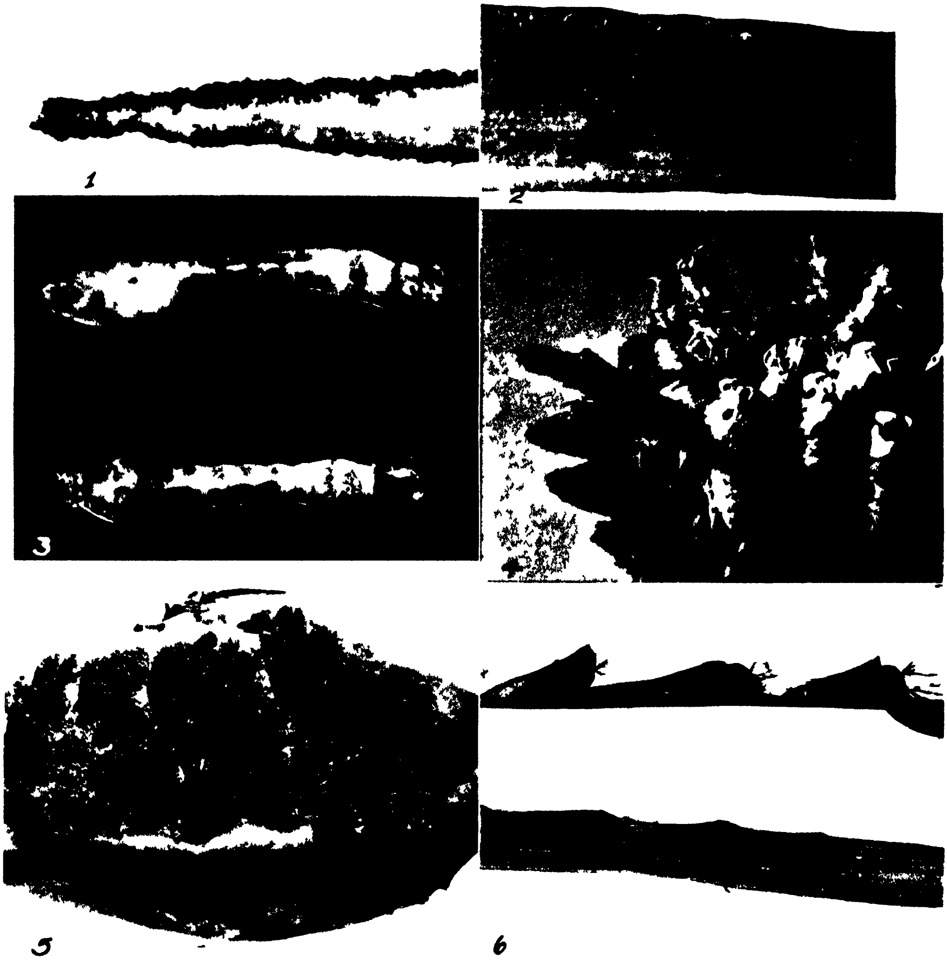
* *Edwards (8) described two South American species in which the eyes of the males are dichoptic and all the facets small.*



1 *Egg masses of S. vittatum Zett. on submerged leaf, about natural size.* 2 *Egg mass of S. venustum Say. X11.* 3 *Mature larvae of S. vittatum Zett., lateral and dorsal views, about six times natural size.* 4 *Pupae and emerging adult of S. lascivum n. sp., about three times natural size.* 5 *Cocoons and pupal skins of S. venustum Say on stone, about natural size.* 6 *Pupae and cocoons of S. vittatum Zett., about 3½ times natural size.*

on South American species, published in 1931. Among American authors, Pomeroy (38) drew attention, in 1916, to the remarkably constant specific characters exhibited by the genitalia of the males; Cameron (2) made use of them, in 1922, in describing the cattle-infesting *S. simile* (= *S. arcticum* Mall.), and Dyar and Shannon (5) largely based their descriptions on the genitalia of the males and females in their revision of the North American species of the family. The last named authors appear to have been the first to use the female terminalia in this connection. Gibbins (15) also described and figured the genitalia of both sexes in a paper on Ethiopian Simuliidae published in 1934.

In describing the genitalia of the species of Eastern Canada included in the present study, I have followed the terminology of Dyar and Shannon. Since the major part of this work was done, a paper by Gibbins (17) on the male terminalia has come to hand. In this he states that the organ situated between the coxites (side-pieces) and referred to by Pomeroy (38), Dyar and Shannon (5), and herein, as the adminiculum is actually the anterior part of the phallosome; the adminiculum arms are the posterior part of that organ.

Classification

Until 1914, the family Simuliidae was considered to consist of only the genus *Simulium*. In that year, Malloch (29) in his study of American blackflies adopted the name *Prosimulium* Roubaud in a generic sense, and set up the new genus *Parasimulium*. Enderlein, in 1921 (10), recognized seventeen genera, and, in 1930 (11), increased this to thirty. Edwards, in 1931 (8), after a study of more than 120 species of Simuliidae in the British Museum, concluded that, with the exception of *Parasimulium*, none of the divisions recognized by other authors should be admitted as full genera, and, at most, seven of them as subgenera: namely, *Prosimulium*, *Morops*, *Gigantodax*, *Austrosimulium*, *Cnephia*, *Eusimulium* and *Simulium*.

The present study of Canadian species has led me to follow Edwards in recognizing as genera only *Parasimulium* and *Simulium*, admitting the others as subgenera of the latter. The genus *Parasimulium* has not been recorded in Canada. Only a single female specimen, of one species, *P. furcatum* Malloch, is known; this was collected in Humboldt County, California, on June 9, 1903, by H. S. Barber.

Three subgenera are represented among the species dealt with here: namely, *Prosimulium* Roubaud, *Eusimulium* Roubaud and *Simulium* Latreille. I have followed Dyar and Shannon (5, p. 12) in placing a fourth, *Cnephia* Enderlein, with the subgenus *Eusimulium*. Three of the species: *mutatum*, *boreale* and *lascivum* have characters of *Cnephia* as outlined by Edwards (8, p. 144); however, to admit it as a subgenus would result in separating the two closely related species, *boreale* and *baffinense*. The characters used to define the subgenera included in this paper are given in the following key.

Key to the Subgenera of *Simulium* in Eastern Canada

1. Radius setose on its entire length 2
   Radius bare between the stem vein and radial sector; sector simple; calcipala and pedisulcus present and distinct; length of hind second tarsus less than twice the width of basitarsus; front usually broad; hairs on costa mixed with spinules . . . . *Simulium* Latreille (p. 131)
2. Radial sector with a long fork; setae on costa not mixed with spinules; calcipala and pedisculcus absent; hind second tarsus much longer than width of basitarsus; front usually broad . . . . *Prosimulium* Roubaud (p. 103)
   Radial sector simple (slightly forked in *lascivum*); setae on costa mixed with spinules; calcipala and pedisulcus present or absent; length of hind second tarsus less than twice the width of basitarsus; front narrowed . . . . *Eusimulium* Roubaud (p. 113)

Key to the Pupae of *Simulium* in Eastern Canada*

1. Cocoon of indefinite form; shapeless, loosely woven 2
   Cocoon of definite form; more or less closely woven 6
2. Pupal respiratory filaments 9; nearly as long as pupa . . . . . *decemarticulatum* n. sp.
   Filaments 12–16, distinctly shorter than pupa 3
3. Filaments 12 (sometimes 13 or 14), arising from two branches, about three-quarters as long as pupa . . . . *mutatum* (Mall.)
   Filaments 14, arising from three branches 4
   Filaments 16, about one-half length of pupa . . . . *hirtipes* Fries
   Filaments more numerous 5
4. Dorsal branch dividing into two stalks, each stalk bearing three filaments; filaments two-thirds length of pupa . . . . *gibsoni* n. sp.
   Dorsal branch dividing into three stalks, each stalk bearing a pair of filaments; filaments two-fifths length of pupa, arctic species . . . . *browni* n. sp.
5. Filaments 23–28; average 26 . . . . *multidentatum* n. sp.
   Filaments 27–40; average 30 . . . . *lascivum* n. sp.
6. Pupal respiratory filaments 4, in two pairs 7
   Respiratory filaments 6 10
   Respiratory filaments more numerous 12
7. Cocoon with long anterior process 8
   Cocoon without such process 9
8. Both pairs of filaments distinctly stalked . . . . *latipes* Meigen
   One pair stalked, the other sessile, or nearly so . . . . *rivuli* n. sp.
9. Both pairs of filaments on short stalks, the lower pair longer than the upper . . . . *quebecense* n. sp.
   Lower pair sessile, upper pair shortly stalked . . . . *aureum* Fries.
10. Cocoon with long anterior process; filaments nearly as long as pupa, in three stalked pairs, the upper pair divergent from the lower pairs . . . . *subexcisum* Edwards
    Cocoon without anterior process 11
11. Respiratory filaments slender, normal, about one-half as long as pupa; cocoon of wall-pocket type, entire . . . . *venustum* Say
    perissum D. & S.
    Filaments stout, inflated, about one-fourth as long as pupa; cocoon slipper-shaped, with an anterior aperture on each side . . . . *fibrinflatum* n. sp.
12. Respiratory filaments 8, six paired, two unpaired; about one-half as long as pupa; cocoon wall-pocket shaped . . . . *ottawaense* n. sp.
    Respiratory filaments 9; about two-fifths as long as pupa; cocoon boot-shaped . . . . *pictipes* Hagen
    Respiratory filaments 10; cocoon slipper-shaped 13
    Respiratory filaments 16; about three-quarters as long as pupa; cocoon wall-pocket shaped . . . . *vittatum* Zett.
    Respiratory filaments approximately 200; short, hairlike, mainly simple, arising from five stout, rootlike tubes spread fingerwise; cocoon boot-shaped . *boreale* (Malloch)
13. Filaments dividing from two short branches, eight paired, two unpaired; about one-fourth as long as pupa, which averages 2.5 mm.; cocoon with anterior aperture on each side . . . . *nigroparvum* n. sp.
    Filaments arising in sessile pairs from a common base; about three-eighths as long as pupa which averages 3.5 mm.; cocoon with anterior apertures and an interlacing basket-like extension . . . . *corbis* n. sp.

***** *Pupa of baffinense unknown; johannseni not included, owing to lack of material: it is apparently similar to aureum, with four filaments in each respiratory tuft.***

Subgenus *Prosimulium* Roubaud

Prosimulium Roubaud, Comp. Rend. Acad. Sci. Paris, 143 : 519, 1906.
Helodon Enderlein, Deuts. Tier, Woch., 29 : 199, 1921.
Taeniopterna Enderlein, Zool. Ans., 62 : 203, 1925.
GENOTYPE.—*Simulium hirtipes* FRIES.

KEY TO THE FEMALES OF *Prosimulium*

(Based on external characters and genitalia)

1. Claws bifid (*i.e.*, with basal tooth); antennae nine or ten-jointed 2
Claws simple; antennae eleven-jointed 3
2. Antennae nine-jointed; tergal plates strongly chitinized, 3, 4, and 5 reduced in width; sternites 2, 3, 4, and 5 membranous; anal lobe sub-quadrate; apical expansions of genital rod transversely wrinkled; upper margins toothed . . . . *gibsoni* n. sp.
Antennae ten-jointed; tergal plates weakly chitinized, 3, 4, 5, 6, and 7 greatly reduced in width; all sternites membranous; anal lobe broadly L-shaped; apical expansions of genital rod smooth, upper margins thickened . . . . *decemarticulatum* n. sp.
3. Tergal plates on segments 3, 4, and 5 greatly reduced; sternites 2, 3, 4, 5, and 6 membranous; anal lobe somewhat L-shaped 4
Tergal plates not greatly reduced; sternites with small chitinized plates; anal lobe triangular; apical expansions of genital rod lanceolate, incurved, inner margins broadly chitinized . . . . *browni* n. sp.
4. Ovipositor reaching tip of abdomen; apical expansions of genital rod quadrate; a large species . . . . *multidentatum* n. sp.
Ovipositor shorter and weaker; apical expansions of genital rod more or less triangular; medium sized species . . . . *hirtipes* Fries.

KEY TO THE MALES OF *Prosimulium**

(Based on genitalia)

1. Claspers with a single terminal tooth; adminiculum arms broad medially 2
Claspers with two or more terminal teeth; adminiculum arms narrow, strap-shaped . 3
2. Setae on side-pieces only on apical margin; claspers of even width throughout; prongs of adminiculum long, blunt; median process pointed at apex . . . . *gibsoni* n. sp.
Setae on side-pieces more generally distributed; claspers sharply tapered; prongs of adminiculum short, pointed; median process broad and flat at apex . . . . *decemarticulatum* n. sp.
3. One clasper with two, the other with three terminal teeth . . . . . . *hirtipes* Fries.
One clasper with four, the other with five terminal teeth . . . . *multidentatum* n. sp.

Simulium (Prosimulium) hirtipes FRIES.

Simulia hirtipes Fries, Mono. Simul. Suec., 1824 : 17.

Female.—Length†, 2–3 mm.; wing, 2.8–3 mm. Integument dark-brown to black; pile pale yellow. *Head:* front moderately broad, distinctly narrowed below; front and clypeus covered with pale yellow pilosity. Antenna 11-jointed, black, two basal joints paler. *Thorax:* mesonotum rather densely covered with pale yellow pile; pleural tuft pale yellow; scutellum with rather long, upright, yellow hairs. *Wings:* brownish; stem vein with pale yellow pilosity, other hairs dark brown; radial sector with a long fork; small basal cell present. *Legs:* brown, the coxae and tarsi darker; pubescence largely pale yellow; fore tarsi cylindrical, fore basitarsus a little shorter than the antenna; calcipala and pedisulcus absent; claws simple. *Abdomen:* basal fringe and short scattered pilosity on all segments, pale yellow; tergites three, four and five distinctly reduced; sternites two to six entirely membranous. *Genitalia* (Fig. 1, A, 5–6): ovipositor well developed, largely membranous, spiculate; inner margins thickly chitinized except the apical third: anal lobe broadly L-shaped, the basal portion equal in length to width of cercus and extending beneath it to the posterior margin; cercus rectangular, a little more than twice as wide as long; both evenly setulose and moderately setose; forks of the genital rod more or less triangularly expanded apically.

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* *Male of browni n. sp. unknown.*

† *The linear measurements of the complete insect of this and all other included species were made from dried pinned specimens.*

Male.—Similar in size, but darker than the female; pubescence on mesonotum coarse, yellow, rather dense, longer on posterior part and scutellum; pilosity on abdomen and legs longer and darker, particularly on the basal scale, venter and hind legs; legs uniformly darker; hind basitarsus more dilated, about equal in width to the tibia; claws bifid; abdominal tergites not greatly reduced; sternites not entirely membranous. *Genitalia* (Fig. 1, A, 1–4): side-piece slightly longer than broad, tapering; claspers shorter than side-piece, conical; one clasper bears two short, stout, terminal teeth, and the other, three (Edwards, 6, p. 42, mentions this character as an abnormality in a British specimen he described, but evidently it is normal for the species); adminiculum broad, membranous, minutely pubescent medially; adminiculum arms smooth, strap-shaped, without teeth.

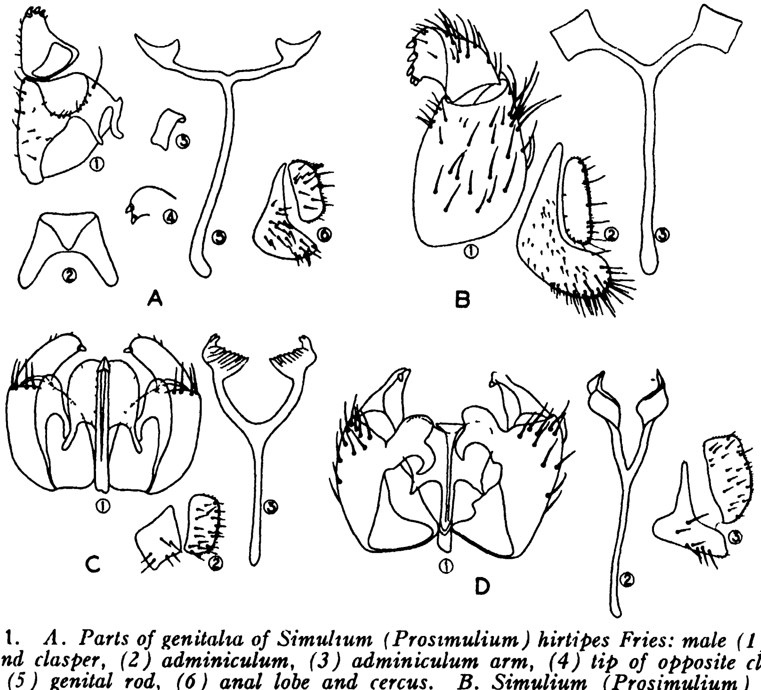

FIG. 1. *A. Parts of genitalia of Simulium (Prosimulium) hirtipes Fries: male (1) side-piece and clasper, (2) adminiculum, (3) adminiculum arm, (4) tip of opposite clasper; female (5) genital rod, (6) anal lobe and cercus. B. Simulium (Prosimulium) multidentatum n. sp.: male (1) side-piece and clasper; female (2) anal lobe and cercus, (3) genital rod. C. Simulium (Prosimulium) gibsoni n. sp.: male (1) side-pieces and claspers, adminiculum and adminiculum arms, median process or penis; female (2) anal lobe and cercus, (3) genital rod. D. Simulium (Prosimulium) decemarticulatum n. sp.: male (1) side-pieces and claspers, adminiculum and adminiculum arms, median process or penis; female (2) genital rod, (3) anal lobe and cercus.*

Pupa.—Length about 5 mm.; the respiratory tuft one-half as long as the pupa, consisting of 16 filaments. This character is constant for all the numerous pupae taken from streams in the Gatineau hills, Que., north of Ottawa, from which adult flies were reared. Various authors: Johannsen (23 and 24), Malloch (29), and others have indicated up to 60 or more respiratory filaments for this species in the United States. Puri (41, p. 362) states that pupae from France attain a total number of 50–60 filaments. He found, however, that pupae from Norway have only 16, and that as the species was originally described from Scandinavia the specimens must be regarded as typical. Puri's description of the filaments of the Norwegian specimens is in perfect agreement with my material and I cannot do better than quote from him: "The first division is near the base into three short stems, each of the two ventral stems dividing almost immediately into two branches, each of which bifurcates again. The upper stem again divides into three branches, the two lower ones dividing into three each and the upper into two. All the filaments are more or less of equal thickness and length." The cocoon consists of a mat of silk of indefinite shape which sometimes only partially covers the pupa, and to which extraneous matter, such as sand and tiny pebbles and fragments of stone is often attached.

Distribution.—Widespread in Europe and North America. Malloch (29, p. 29) and Dyar and Shannon (5, p. 9) give records from several of the states of the Eastern United States, and Labrador and Newfoundland. Dyar and Shannon state that the species appears to be confined to the region east of the Mississippi river and north of the Carolinas, but Malloch also includes records from Arizona, Idaho and British Columbia. Hearle (20, p. 8) records it from Banff, Alberta. It is possible that the western records concern allied species.

Simulium hirtipes occurs widely in Eastern Canada. I have examined specimens from the following localities. *Nova Scotia:* Kentville, 24. V. and 13. VI. 23 (R. P. Gorham); *New Brunswick:* Fredericton, 21. VI. 13 (collector unknown), Barber Dam, 23. VI. 14 (J. D. Tothill); *Quebec:* Grand Cascapedia, Gaspé, 16. IX. 32 (M. L. Prebble), Federal Mine, Cascapedia (2000') 4. X. 32 (R. E. Balch), Gaspé, 26. VII. 33 (E. B. Watson), Hemmingford, 31. V. 26 and 25. VII. 28 (G. H. Hammond), St. Hilaire, 24. V. 06 (Beaulieu), Miners Bay, 26. V. 27 (F. P. Ide), Montebello, 21–31. V. 30 (N. J. Atkinson), Kingsmere, 14. V. 22 (J. McDunnough), Meach Lake, 24. V. 32 (C. R. Twinn), Hull, 22. V. and 10. VI. 23 (C. H. Curran), Chelsea, 5. VI. 19 (Arthur Gibson); *Ontario:* Ottawa, 13. V. 22 (J. McDunnough), Algonquin Park, 17. V. 32 (C. R. Twinn). I have also collected the immature stages and reared and collected adults in the Gatineau hills, Que., north of Ottawa, on several occasions in recent years.

Notes.—In the Ottawa district, the larvae and pupae of *S. hirtipes* have been found only in cold streams flowing down wooded hillsides from small lakes, in the Gatineau hills (Laurentians), Que. In one of these streams (Fig. 2, A), pupae were found on May 2, 1935, when the temperature of the water was 47° F.; adults emerged indoors on May 4. Even in July, I found the temperature of this stream to be only 62° F. (July 22), but on that date *hirtipes* was replaced by *venustum*. Smart (49, p. 24) who gives the type locality as Lapland, states that *hirtipes* is typically a northern species, and an inhabitant of hill streams at Fortingal, in Scotland. In Eastern Canada, the adults are numerous in hilly, forested country in early spring, and may become abundant again in the autumn. I have found the pupae only in spring, and the species appears to have but one generation; overwintering in the larval stage. The females attack animals and man. Specimens of troublesome blackflies sent in by field officers of the Dominion Entomological Branch, from points in the forested region of New Brunswick and the Gaspé peninsula, have been this species and *S. venustum*.

Simulium (Prosimulium) multidentatum N. SP.

Female.—Length 2.7–3.5 mm.; wing 3.5–3.9 mm. closely allied to *hirtipes* Fries, but of more robust appearance; legs darker; general pilosity paler and more conspicuous; fore basitarsus slightly shorter than antenna (differs from *magnum* D. & S.). *Genitalia* (Fig. 1, B, 2–3): similar to, but larger and stronger than *hirtipes;* ovipositor chitinized, strigate, reaching tips of anal lobes which in turn extend beyond the cercus, and are strongly setose along the postero-ventral margin; cercus much wider than long, with elongate setae on the posterior margin; arms of genital rod quadrately expanded.

Male.—Essentially as in *hirtipes*, but more robust, and easily separated from it, and from *magnum*, by the number of teeth at the tip of the claspers. *Genitalia* (Fig. 1, B, 1): one clasper bears five short, stout, terminal teeth, the other four (the number being constant in several specimens examined); in other respects the genitalia are similar to *hirtipes*.

Holotype.—Male, near Hull, Que., reared from pupa, 3. V. 35. Can. Nat. Coll. (slide).

Allotype.—Female, same data.

Paratypes.—Eleven males, 13 females, 1–3. V. 35.

Pupa.—Length 5–6 mm. Respiratory tuft about one-third as long as pupa, comprised of 23–28 filaments (counts of six specimens gave 23, 25, 25, 26, 27, 28; an average of 26) branching from the main trunk, close to the base. The cocoon is indefinite in shape and dirty-gray in color. The pupae occur in close masses on submerged obstacles such as rocks, stones, pieces of wood and other debris.

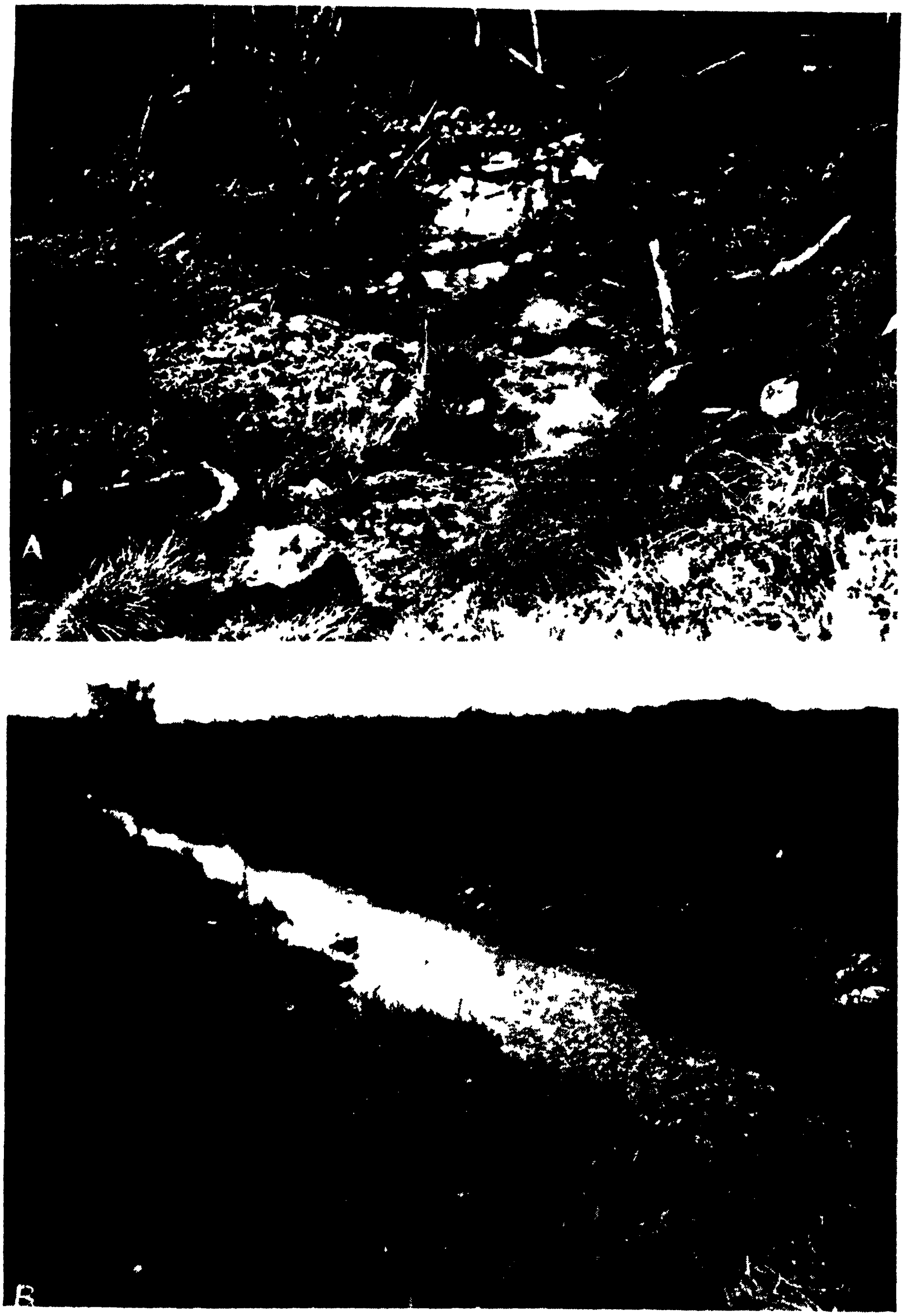

FIG. 2. *A. Stream (M.R. 1) in Gatineau hills near Hull, Que., habitat of S. hirtipes Fries and S. venustum Say. B. McEwan Creek, near Ottawa, Ontario, breeding place of S. vittatum Zett., S. venustum Say, and S. aureum Fries.*

Notes: Gross infestations of larvae and pupae of multidentatum were found on April 29, 1935, in two small, shallow, more or less parallel streams (Fig. 3, A and B), a few miles in length, which have their source south of the Gatineau hills and flow through agricultural land, finally emptying in the Ottawa river, near Hull, Que. The temperature of the water was 52° F. in one, and 54° F. in the other. No empty pupal skins were seen, but large numbers of adults of both sexes emerged between May 1 and 3 from pupae placed in moist vials at room temperatures.

A goodly proportion of the larvae were parasitized by nematodes. Infested individuals had distended abdomens and undeveloped histoblasts. The nematodes were coiled up in the abdomen; some of them measured more than an inch in length (one was 39 mm.), or three to four times as long as their hosts. An examination of 276 larvae showed 63, or 22.8%, parasitized. The nematodes left the larvae shortly after the latter were removed from the water. This may be the same species of parasite that Strickland (51, 52) recorded, in 1911, from larvae of *S. hirtipes* taken in streams near Boston, Mass. He concluded (51, p. 326) that the parasite, *Mermis* sp., "does not affect the larval development to any extent, except by slightly increasing its size, but it inhibits the development of the histoblasts to such an extent that pupation becomes impossible."

Emergence of the adults in the field had commenced on May 8, and was practically complete by May 14. No signs of the larvae were found throughout the summer, but their place was taken by sparse numbers of *vittatum*, *venustum* and *aureum*. Pupae and larvae which I collected on May 17, 1932, from Cranberry creek, Algonquin Park, Ontario, proved to be *multidentatum*.

Simulium (Prosimulium) gibsoni N. SP.

Female.—Length 1.6–1.9 mm.; wing 2.2–2.4 mm. Integument dark-brown to black; vestiture pale yellow. *Head:* front moderately broad, divergent-sided; front and clypeus with scattered pale hairs. Antenna dark reddish-brown; 9-jointed; a little shorter than the fore basitarsus; joints subequal, gradually tapering to the apex; joints eight and nine distinct, but incompletely separated; palpi black. *Thorax:* pile pale yellowish, the long hair on and in front of the scutellum also pale. *Wings:* with the characters of the subgenus; stem vein pale pilose; a few pale hairs mixed with the dark hairs elsewhere on the veins; halteres brown. *Legs:* dark-brown, distal segments of tarsi almost black; hairs pale-yellowish, especially long and conspicuous on the fore femora and hind legs; claws with a pointed basal tooth. *Abdomen:* basal fringe and short, rather sparse general pilosity, pale yellow; plates on the dorsum strongly chitinized, those on segments three, four and five reduced in width; sternites two to five largly membranous. *Genitalia* (Fig. 1, C, 2–3): anal lobe with few setae, small, more or less quadrate, slightly produced antero-dorsally; cerci rectangular, outwardly rounded, about twice as wide as long, moderately setose; arms of genital rod with apical membranous expansions transversely ridged or fluted, the upper margin crenulate.

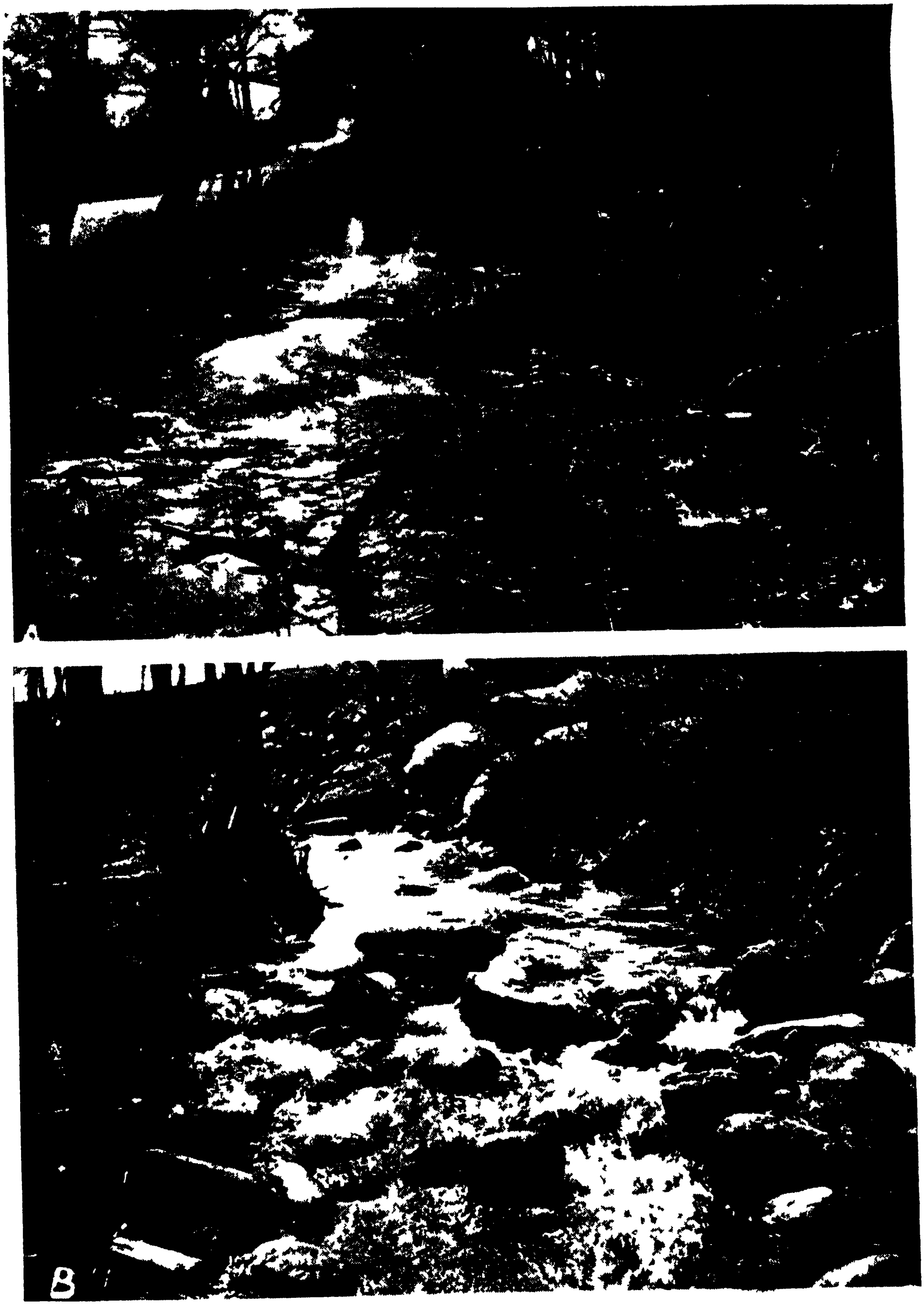

Fig 3 *A Simmons creek, near Hull, Que, breeding place of S multidentatum n sp, S vittatum Zett, and S venustum Say B Fairy Lake stream, near Hull, Que, breeding place of the above species and S aureum Fries*

Male.—Similar to female in size and color; pilosity somewhat longer; antennal joints eight and nine less distinctly separated; hind basitarsus equal in width to the tibia; tergal plates of abdomen not reduced; sternal plates weakly chitinized. *Genitalia* (Fig. 1, C, 1): side-pieces longer than broad, gently tapering, smooth, with a row of setae on the apical margin. Claspers a little longer than the side-pieces, with few setae and a short, stout, apical tooth. Adminiculum somewhat membranous, about as broad as long, setulose throughout, basal prongs chitinized, slender, divergent; adminiculum arms without teeth, broad medially and narrowed at both ends. Median process (penis ?) pointed at tip.

Holotype.—Female, near Carleton Place, Ont., emerged from pupa, 10. V. 35. Can. Nat. Coll.

Allotype.—Male, same data, 9. V. 35.

Paratypes.—Five females, 2 males, near Hull, Que., reared from pupae, 21–23. V. 35.

This species is dedicated to Dr. Arthur Gibson, Dominion Entomologist, whose kind approval made the present study possible.

Pupa.—Length about 3.8 mm. Respiratory tuft about two-thirds the length of pupa and comprised of 14 filaments, which divide from three branches, one dorsal and two ventral, arising from the main trunk. The dorsal branch divides into two, each part bearing three filaments, a single one and a short-stalked pair, thus forming six filaments; the ventral branches each bifurcate twice making eight filaments; or 14 in all. The cocoon, in which the pupa lies partially or wholly concealed, has no definite shape, and consists of loosely woven silk encrusted with tiny particles of sand. Sometimes the pupa is nearly naked.

Notes.—Larvae and pupae were collected during May, 1935, with other species, from three small, shallow, temporary streams draining pasture land near Hull, Que., and Carleton Place, Ont. A male emerged on May 9, and a female on May 10, from pupae taken on May 8, from the two streams near Carleton Place (C.P.H. 1 & 2: Fig. 4, A and B). Two males and five females emerged May 21-23, from pupae taken on May 19, on stones and pebbles in the stream (S. R. S. 1) near Hull, Que. Specimens were not seen during subsequent examinations, and the streams were dry in August. Numbers of empty pupal skins of *gibsoni* were found in the Blanche river, south of Perkins Mills, Que., on May 22. This is an early spring species with probably only one generation annually.

Simulium (Prosimulium) decemarticulatum N. SP.

Female.—Length about 2 mm.; wing 3 mm. A species with dark-brown integument, yellowish pilosity and yellowish-brown legs. *Head:* front moderately broad, distinctly narrowed below; surface hairs on front and clypeus

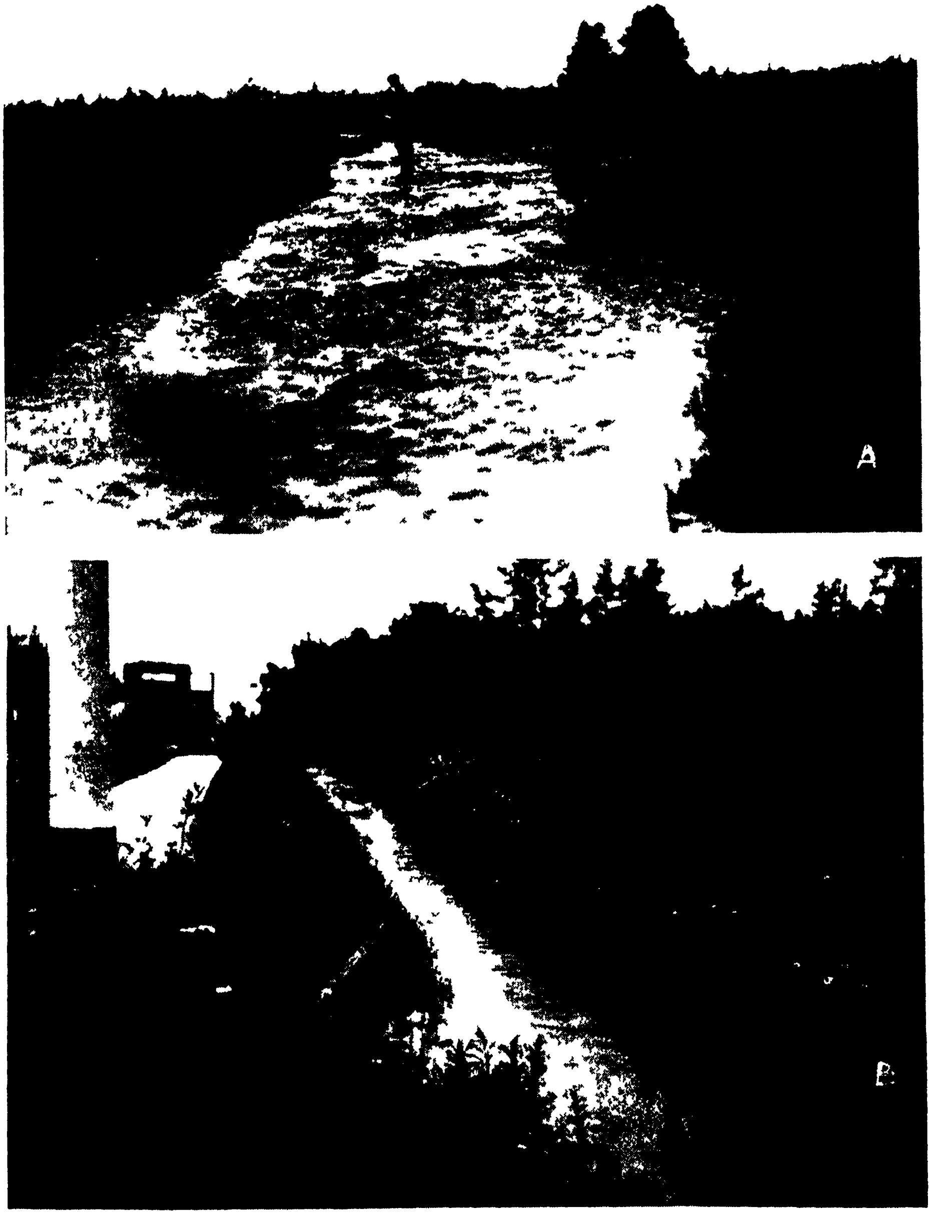

FIG. 4. *A. Stream (C.P H. 2) near Carleton Place, Ont., habitat of S. gibsoni n sp., S. decemarticulatum n. sp., S. mutatum Mall., S. subexcisum Edw., S. venustum Say, B. Roadside ditch (C.P H. 1) near Carleton Place, Ont., breeding place of the above species and S. rivuli n. sp.*

largely pale, yellowish, postocular hairs black. Antenna 10-jointed; about one-third longer than the fore basitarsus; brown, basal joints paler; joints sub-equal, gradually tapering. *Thorax:* mesonotum rather densely covered with yellowish pubescence; the longer, upright hairs on and in front of scutellum, yellow; pleural tuft yellow. *Wings:* hairs on the stem vein pale yellow; elsewhere on the veins, dark; radial sector with a long fork; small basal cell present; halteres pale-brown. *Legs:* yellowish-brown, apical tarsal segments darker; pilosity yellow; calcipala and pedisulcus absent; claws bifid. *Abdomen:* basal fringe yellow; pilosity on the dorsum pale, sparse, denser at the sides; tergites weakly chitinized, the plates on segments three to seven greatly reduced in width; sternites entirely membranous. *Genitalia* (Fig. 1, D, 2–3): ovipositor valves weakly chitinized, extending slightly beyond base of anal lobe; cercus evenly and moderately setose, rectangular, twice as wide as long, the postero-ventral angle cut off by the inwardly directed posterior margin; anal lobe L-shaped, the broad basal portion nearly twice the length of the cercus, sparsely setose; upper arm narrow, thinly chitinized, bare; arms of genital rod narrowly divergent, expanded portions with upper margins thickened.

Male.—Similar in size to the female, but distinctly darker; integument dark-brown to black; mesonotal pile golden; pleural tuft black; hairs on scutellum dark; legs dark-brown, pilosity brown to black, in some places intermixed with paler, golden hairs, especially on femora; hind basitarsus dilated to about the width of tibia. *Genitalia* (Fig. 1, D, 1): similar to *gibsoni*, but side-pieces more setose; claspers sharply tapering; adminiculum broader than long, chitinized basal prongs very short and tapered; median process broad and flattened at apex.

Holotype.—Female, near Carleton Place, Ont., emerged from pupa 10. V. 35. Can. Nat. Coll.

Allotype.—Male, same data, 8. V. 35.

Paratypes.—Two females, same data, 9. V. and 13. V. 35.

Pupa.—Length about 4 mm. The respiratory tuft consists of nine long filaments, the longest nearly equalling the length of the pupa, arranged in a whorl, and bent almost at right-angles to the short base. The cocoon is of the usual *Prosimulium* type; shapeless and loosely woven. Those collected were encrusted with sand particles, which made the insect difficult to find.

Notes.—Larvae and pupae were found with immature stages of other species, in two shallow, temporary streams (Fig. 4, A and B) near Carleton Place, Ont., on May 8. One of the streams was a pebbly-bottomed, roadside drainage ditch, and the other flowed over a rock bottom through pasture fields. This is evidently an early spring species; no specimens were found two weeks later, and the stream dried up during the summer.

Simulium (Prosimulium) browni N. SP.

Female.—Length 2.5–3 mm.; wing 3.3–4 mm.; integument black, pilosity pale, yellowish. *Head:* front and clypeus black, rather sparsely clothed with black and pale hairs intermixed; front very broad, moderately narrowed below. Antenna 11-jointed, entirely black, distinctly longer than head and about one-third longer than the fore basitarsus; three basal segments enlarged, remainder smaller, tapering. *Thorax:* dull-black, concolorous; mesonotum with pale, sparse, recumbent pile; pleural tuft yellowish-brown, inconspicuous; marginal fringe of scutellum yellowish-brown, moderate. *Wings:* with a brown tinge; anterior veins brown; stem vein pale pilose; radial sector with a long fork; minute basal cell present; halteres dark-brown. *Legs:* dark-brown to black; pilosity pale yellowish, moderate; calcipala and pedisulcus absent; claws simple. *Abdomen:* black, basal fringe dark-brown; short, sparse, pale hairs on the tergites, longer and denser at the sides; tergites not markedly reduced; sternites all with small chitinized plates. *Genitalia* (Fig. 5): ovipositor valves similar to *hirtipes*, but infuscated, the setae longer, more numerous, basal half of inner margin chitinized, incurved; anal lobe scarcely larger than cercus, with numerous long setae on the ventral half; cercus rectangular, twice as wide as long, rounded distally, setose; genital rod stout, strongly chitinized, the arms broadly expanded, incurved.

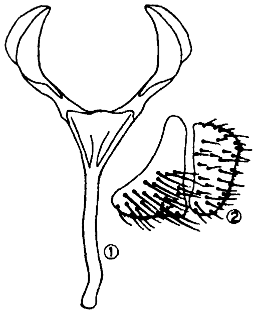

FIG. 5. *Parts of female genitalia of Simulium (Prosimulium) browni n. sp.: (1) genital rod, (2) anal lobe and cercus.*

Holotype.—Female, Lake Harbour, Baffin Island, 10. VIII. 35, reared from pupa (W. J. Brown). Can. Nat. Coll.

Paratypes.—Eleven females, same data.

Pupa.—Length 4.5 mm. Respiratory tuft about two-fifths as long as pupa, consisting typically of 14 filaments (one specimen had 15). The short main trunk gives rise to three branches, one dorsal and two ventral. The dorsal branch divides into three stalks, each of which bifurcates forming six filaments. The two ventral branches each give rise to four filaments, making 14 in all. The cocoon is of the usual type; a rather loosely woven sheath of indefinite shape. When undamaged it largely conceals the pupa.

Notes.—W. J. Brown, of the Dominion Entomological Branch, Ottawa, collected larvae and pupae from a stream at Lake Harbour, and reared the adults which comprise the type material. He also collected nine females on the wing, 5–10. VIII. 35. The species is dedicated to him.

Subgenus *Eusimulium* Roubaud

Eusimulium Roubaud, Comp. Rend. Acad. Sci. Paris, 143 : 519, 1906.

Cnephia Enderlein, Deuts. Tier. Woch., 29 : 199, 1921.

Cnetha Enderlein, ibid.

Nevermannia Enderlein, ibid.
Schonbaueria Enderlein, ibid.
Pseudonevermannia Baranoff, Neue Beitr. Syst. Ins., 3 : 164, 1926.
GENOTYPE.—*Simulium aureum* FRIES.

KEY TO THE FEMALES OF *Eusimulium**

(Based on external characters and genitalia)

1. Radial sector simple 2
   Radial sector weakly furcate 7
2. Claws simple 3
   Claws bifid (*i.e.*, with basal tooth) 4
3. Pedisulcus present, distinct; calcipala small; hind tibial spurs normal; thorax with three faint mesonotal vittae; hairs on stem vein largely yellow; anal lobe small, subquadrate, with a distal, rounded, setose protuberance; arms of genital rod quadrately expanded (arctic species) . . . . *baffinense* n. sp.
   Pedisulcus absent; calcipala prominent; hind tibial spurs unusually long; mesonotum unstriped; hairs on stem vein black; anal lobe narrowed behind cercus, ventrally expanded, lobed beneath; arms of genital rod quadrately expanded, irregularly toothed . . . . *mutatum* (Mall.)
4. Postnotum with patch of yellow scales; mesonotal pubescence brassy-yellow, scale-like; two basal joints of antennae pale-brown; anal lobe small, narrow, curved beneath cercus, ventral margin setose . . . . *aureum* Fries
   Postnotum without scales; mesonotal pubescence pale; antennae entirely black . . 5
5. Mesopleural membrane with a tuft of pile; calcipala and pedisulcus absent; anal lobe normal, posteriorly setose . . . . *boreale* (Mall.)
   Mesopleural membrane bare 6
6. Calcipala prominent; pedisulcus deep; hind basitarsus about 7.5 × 1; anal lobe normal; arms of genital rod irregularly expanded and terminating in a blunt chitinized tooth . . . . *quebecense* n. sp.
   Calcipala small; pedisulcus shallow; hind basitarsus about 6 × 1; anal lobe subquadrate, the distal margin with a rounded, chitinized, setose process; arms of genital rod quadrately expanded . . . . *subexcisum* Edw.
7. Calcipala minute; pedisulcus shallow; claws with small, sub-basal tooth; anal lobe elongate quadrangular, strongly setose; expansions of genital rod toothed and ribbed . . . . *lascivum* n. sp.

KEY TO THE MALES OF *Eusimulium*†

(Based largely on genitalia)

1. Adminiculum narrow, conical; basal prongs widely divaricate . . . . . *aureum* Fries
   Adminiculum broad, rounded; basal prongs incurved, or only slightly divergent . . 2
2. Adminiculum with hirsute nipple in medial depression; adminiculum arms each with a single long, stout tooth . . . . *quebecense* n. sp.
   Adminiculum without hirsute nipple; adminiculum arms with few to many, smaller teeth 3
3. Clasper with a pair of close-set terminal teeth . . . . *mutatum* (Mall.)
   Clasper with a single terminal tooth 4
4. Radial sector weakly furcate; basal prongs of adminiculum roundly expanded . . . . *lascivum* n. sp.
   Radial sector simple; basal prongs not rounded 5
5. Mesopleural membrane with a tuft of pile . . . . *boreale* (Mall.)
   Mesopleural membrane bare 6
6. Pedisulcus deep; upper margin of adminiculum depressed; arms with numerous small teeth; large (arctic) species . . . . *baffinense* n. sp.
   Pedisulcus shallow; upper margin of adminiculum convex; arms with few teeth; smaller species 7
7. Hind basitarsus about 6 × 1; adminiculum minutely hirsute, basal prongs incurved; arms with short stout teeth . . . . *subexcisum* Edw.
   Hind basitarsus about 5 × 1; adminiculum with longer hairs, basal prongs not incurved; teeth on arms larger . . . . *rivuli* n. sp.

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* ***Female of rivuli unknown; latipes and johannseni not included.***
† ***Latipes and johannseni not included.***

Simulium (Eusimulium) aureum Fries

Simulia aureus Fries, Monogr. Simul. Suec., p. 16, 1824.

Simulium bracteatum Coquillett, U.S.D.A. Bur. Ent. Bull. 10, n.s. p. 69, 1898.

Simulium angustipes Edwards, Bull. Ent. Res. 6 : 40, 1915.

Female.—Length, 2–3 mm.; wing, the same; integument black, grayish to brownish pollinose; pubescence silvery to brassy yellow. *Head:* front narrow, sides divergent; pilosity scale-like, creamy-yellow; clypeus distinctly longer than broad, pile finer and looser than on front; post-ocular hairs numerous, scale-like, pale yellow. Antenna 11-jointed, hardly tapering; black, the two basal joints pale-brown; palpi black. *Thorax:* mesonotum rather densely clothed with a yellow or brassy scale-like pile, somewhat silvery on the anterior margin; the recumbent pile and upright hairs on the scutellum, and a patch of scales on the postnotum, yellow; pleural tuft pale yellowish. *Wings:* hairs on the stem vein and base of costa pale-yellow; other hairs black, those on the costa mixed with minute spinules; the basal two-thirds of sub-costa with a sparse row of hairs. The radial vein hairy on its entire length, the hairs in a single row apically, interspersed with spinules. The radial sector simple; basal cell absent; halteres pale, the stem darker. *Legs:* pale-brown, except

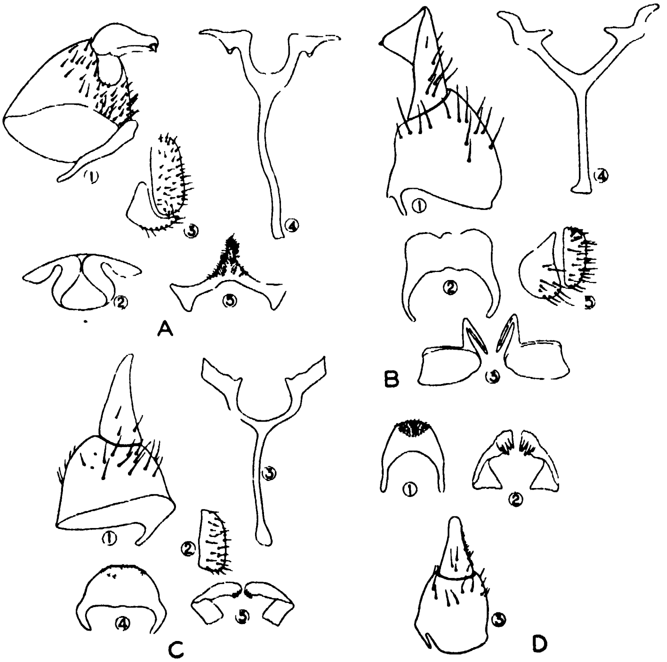

FIG. 6. *A. Parts of genitalia of Simulium (Eusimulium) aureum Fries: male (1) side-piece and clasper, (5) adminiculum, (2) adminiculum arms; female (3) anal lobe and cercus, (4) genital rod. B. Simulium (Eusimulium) quebecense n. sp.: male (1) side-piece and clasper, (2) adminiculum (hairs omitted from nipple), and (3) adminiculum arms; female (4) genital rod, (5) anal lobe and cercus. C. Simulium (Eusimulium) subexcisum Edwards: male (1) side-piece and clasper, (4) adminiculum, (5) adminiculum arms; female (2) cercus, (3) genital rod. D. Simulium (Eusimulium) rivuli n. sp.; male (1) adminiculum, (2) adminiculum arms, (3) side-piece and clasper.*

the mid and hind coxae, the apices of the femora and tibiae, and the tarsal joints, which are black. Pilosity on the pale parts silvery to yellowish; that on the dark parts black. Fore basitarsus a little shorter than antenna; hind basitarsus 6 × 1, about four-sevenths as wide as tibia; calcipala and pedisulcus distinct; claws distinctly bifid. *Abdomen:* basal fringe pale yellow; general pilosity rather dense, scale-like, yellow, sometimes silvery. The chitinized plates of tergites three to seven markedly reduced; sternites one to six membranous. *Genitalia* (Fig. 6, A, 3–4): ovipositor valves weakly chitinized, conical, pointed, reaching posterior margin of anal lobe; setose, spicular on distal two-thirds. Anal lobe smaller than cercus, curved, narrowed to a rounded point beneath cercus, but not reaching distal margin; setose on posterior margin only. Cercus slightly more than twice as wide as long, distally rounded, setose. Arms of genital rod broadly expanded, a blunt chitinized tooth on the outer margins.

Male.—Similar to the female except as follows: antenna more slender and entirely black; mesonotum densely clothed with golden pubescence, except the disc which is velvet black; halteres darker; legs as in female, but the pale parts a little darker, and the pilosity somewhat longer, particularly on the hind legs; the hind basitarsus about 5 × 1; narrower than the tibia; abdomen velvet black, the yellow pilosity less generally distributed, and rather longer and more hair-like; short and sparse on the apical segments; tergal plates not markedly reduced; chitinized sternal plates present on all but the second segment. *Genitalia* (Fig. 6, A, 1, 2, 5): side piece conic-quadrate; clasper small, angled, terminating with a short tooth. Adminiculum narrow, somewhat conical, hirsute, the basal prongs widely divaricate and outwardly triangularly expanded. Adminiculum arms connected medially with a convex membrane; lateral chitinous plates broad.

Pupa.—Length about 3 mm.; the respiratory filaments a little longer than the pupa, four in each tuft, all of about equal thickness, arising in two pairs; the lower pair directly from the main trunk, and the upper pair on a very short stalk. The cocoon is of rather tightly woven silk, of the typical wall-pocket form; about one-third longer than wide. The thickened anterior margin bulges to form a slight median salient.

Synonymy.—Dyar and Shannon (5, p. 14) placed *bracteatum* Coquillett as a race of the European *aureum* Fries. Edwards (7, p. 243) stated that he examined a male of *bracteatum* from Spartanburg, S.C., and could find no appreciable difference from the European *aureum* in the genitalia or other characters; furthermore, descriptions of the larvae and pupae by Strickland (50) and Jobbins-Pomeroy (38, p. 13) also agreed with the British form. After careful comparison of my material with Edwards' descriptions of the male and female of *aureum* (6, pp. 39–40), the former under the synonym *angustipes*, and with descriptions of the larvae and pupae by Edwards (7, p. 242) and Puri (41, p. 355), I am convinced that the North American form is identical with the European *aureum* and have placed *bracteatum* as a synonym of the former.

Notes.—I have collected the larvae and pupae in several localities in Ontario and Quebec, in the Ottawa region, always in small, shallow streams, and usually associated with *venustum* and *vittatum*, but never very numerous. The type of stream (Fig. 2, B and Fig. 3, A) favored, commonly has a stony, pebbly, or shaly bottom, and is usually temporary or semi-permanent in character. There are probably two, perhaps three, generations annually. Pupae were found in June, July, and September. Both sexes were reared from Pink's Lake stream, near Hull, Que., Sept. 25, 1934, and June 12, 1935, and from McKay Lake stream, Rockcliffe, Ont., June 28, and July 24, 1935. Apparently nothing is known of the habits of this species as a bloodsucker.

Distribution.—The records given by Dyar and Shannon (5, p. 14) and by Malloch (29, p. 38) under the name *bracteatum* show this species to occur widely in the eastern United States. It is also recorded from the west by the same authors (Alaska, California and Colorado). Edwards (8, p. 150) states that it occurs throughout Europe and North America. I have the following records for Canada; the collections were pupae unless otherwise indicated. *Ontario:* Bowesville, June 12, 1926; Billings Bridge, Sept. 23, 1926; Rockcliffe, Sept. 27, 1926; June 2, 1931 (reared adults, June 28 and July 24, 1935); Hawkesbury (larvae), July 25, 1927. *Quebec:* Gracefield, July 13, 1926; Luskville, July 22, 1931; Pink's Lake (near Hull), July 21, 1931 (reared adults Sept. 25, 1934 and June 12, 1935). The species is probably widespread in Eastern Canada.

Simulium (Eusimulium) quebecense N. SP.

Female.—Length about 1.6 mm.; wing 2.6 mm.; integument black with grayish pollinosity; vestiture pale. Closely related to *aureum* Fries, with identical characters except as follows: pubescence paler throughout, silvery to yellowish-white or gray, less scale-like; antenna entirely black; postnotum bare; hairs on stem vein pale; legs more slender and darker, the apical bands on the femora and tibiae indistinct; even in cleared specimens; pilosity on the legs pale, the hairs darker on the tarsal joints; hind basitarsus 7.5 × 1; abdominal pubescence pale and much sparser. *Genitalia* (Fig. 6 B, 4–5): ovipositor valves membranous, chitinized on the inner margins, tips not reaching ninth sternite. Anal lobe about twice as long as cercus, broadly rounded, apically produced, pyriform, with sparse long setae postero-ventrally; cercus a little more than twice as wide as long, setose. Genital rod widely forked, each arm irregularly expanded and terminating with a blunt chitinized tooth.

Male.—Slightly larger than female; integument and pubescence much darker; antennae and palpi more slender. Mesonotum velvet black, with pale-yellowish rather scale-like pilosity, the hairs most numerous anteriorly, sparse on the disc; recumbent scales on the scutellum pale, upright hairs black; pleural tuft black. Hairs on the stem vein black; halteres black. Legs dark-brown to black, the pile dark and rather long, especially posteriorly on the front femora and hind legs; hind basitarsus broad, about 3.5×1, six-sevenths the width of the tibia; basal tooth on claws somewhat smaller. Abdomen velvet brown, almost black; other pubescence the same, but short and sparse on the dorsum, long on the sides and sternites. Tergal plates two to six slightly reduced; second sternite entirely membranous. *Genitalia* (Fig. 6, B, 1–3): side-piece conic-quadrate; clasper about as long as side-piece, apical portion conically produced at right-angles to the main axis, and with a small terminal tooth. Adminiculum broad, the centre of the upper margin depressed and bearing a rounded hirsute nipple; basal prongs long, chitinized, incurved. Adminiculum arms rounded-quadrate basally, each bearing a single long, stout, pointed tooth.

Holotype.—Female, near Perkins Mills, Que., 26. V. 35 (reared). Can. Nat. Coll.

Allotype.—Male; same data.

Paratypes.—Four females, five males; same data.

Pupa.—The pupa and cocoon closely resemble those of *aureum* Fries, but both pairs of filaments are arranged on short stalks arising from the main trunk, the lower stalk a little longer than the upper stalk: the uppermost filament slightly swollen at the base. The cocoon is more tightly woven, and the anterior margin thicker.

Notes.—Pupae from which the type material was reared were collected on May 22, 1935, from stones in the rapids of the Blanche River, a short distance below the falls, about five miles south of Perkins Mills, Que. (Fig. 7, A). The temperature of the water was 53° F. At this point the river is shallow and swift, over a stony bottom. A pupal skin of *hirtipes*, some mature larvae of *venustum*, and a number of pupae of *corbis*, were taken at the same time, the latter on submerged dogwood.

Simulium (Eusimulium) subexcisum EDWARDS

Simulium subexcisum Edwards, Bull. Ent. Res. 6 : 41, 1915.

Female.—A small, dark-colored species, integument brown to black; pubescence pale. *Head:* antenna entirely black, 11-jointed; second joint swollen, about equal in length to third joint, remainder smaller, sub-equal, gradually tapering to a point. *Wings:* hairs on apical half of costa mixed with spinules; an irregular row of hairs present on the basal two-thirds of the subcosta; radial vein clothed with fine hairs throughout its length, the apical ones in a single row interspersed with several spinules; radial sector simple; there is a slight but perceptible thickening of the vein, and a slight divergence of the two rows of hairs at the apical margin. *Legs:* all the joints in the cleared specimen uniformly dark; fore and mid basitarsi cylindrical; hind basitarsus flattened, about 6 × 1 and three-fifths width of tibia; calcipala small, narrow; pedisulcus shallow; claws with a prominent basal tooth. *Abdomen:* width of tergal plates three to seven much reduced; sternites largely membranous. *Genitalia* (Fig. 6, C, 2–3): anal lobe small, sub-quadrate, weakly chitinized; ventral margin curved, minutely nodulose, spiculate; distal

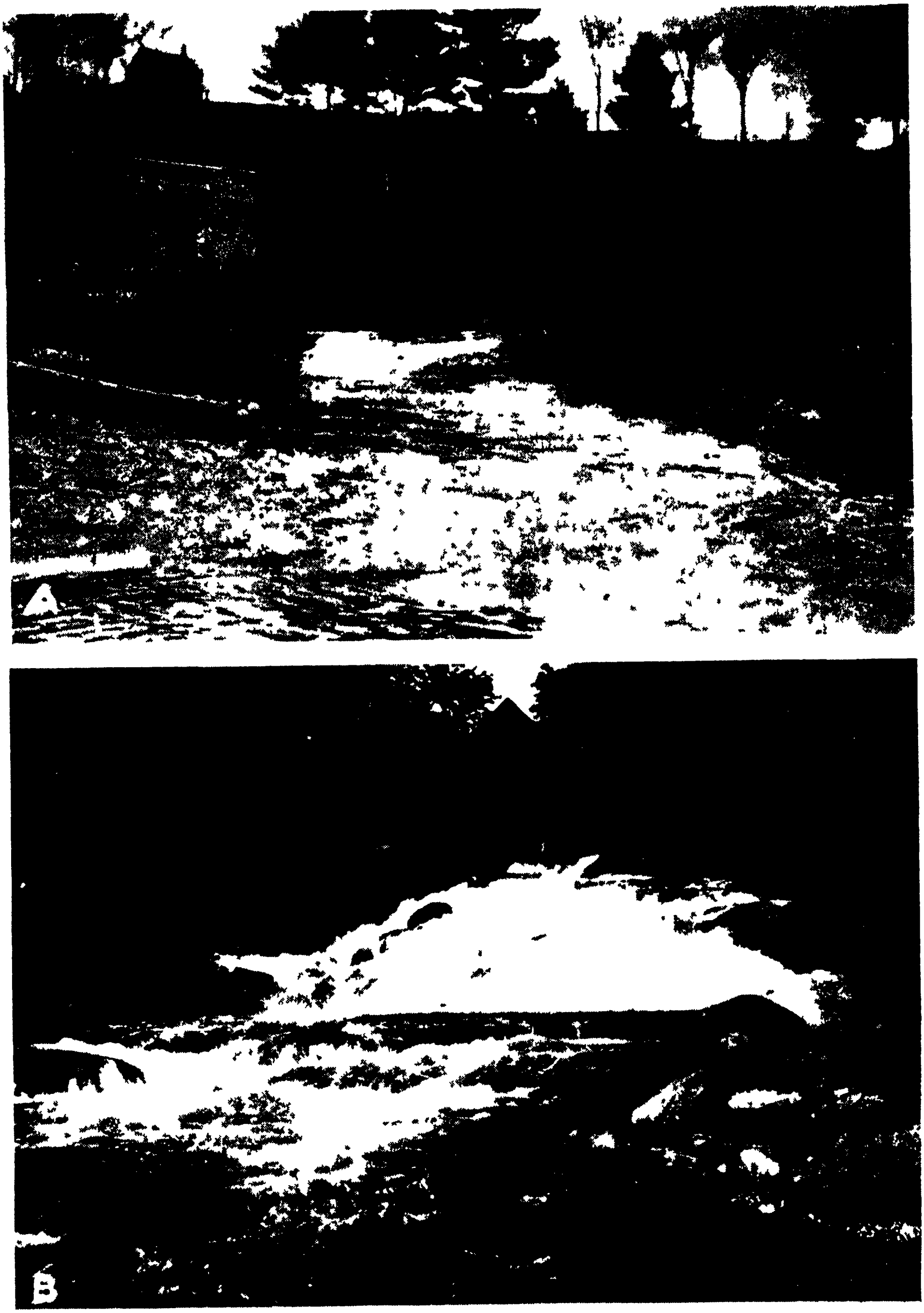

FIG. 7. *A. Blanche river south of Perkins Mills, Que. S. gibsoni n. sp., S. venustum Say, S. quebecense n. sp., S. corbis n. sp. and S. nigroparvum n. sp. breed here. B. Falls of Blanche river at Perkins Mills, Que., breeding place of S. pictipes Hgn.*

margin ventrad of cercus, with a rounded chitinized process bearing two or three long setae. Cercus wider than long, distally rounded, moderately setose. Arms of genital rod quadrately expanded outwardly, the expanded portion weakly chitinized and with the upper-inner margin drawn into a small pointed tooth-like process.

Male.—Differs from female as follows: maxillary palpi markedly shorter than antennae; row of hairs on sub-costa absent; legs in cleared specimen paler, apices of hind and mid femora and all tibiae distinctly darkened; hairs on front femora, hind femora and tibiae longer; abdominal tergites not greatly reduced; only second sternite entirely membranous. *Genitalia* (Fig. 6, C, 1, 4, 5): side-piece conic-quadrate, about as broad as long. Clasper as long as the side-piece, tapering, pointed, with a very small terminal tooth. Adminiculum broadly rounded, minutely hirsute, the basal prongs stout, chitinous, incurved. Adminiculum arms with a group of several short, rather stout teeth.

Pupa.—Length about 3 mm. Respiratory tuft almost as long as the pupa, consisting of six filaments arranged in pairs; the upper pair with a long stalk, widely divergent from the two lower pairs. The cocoon is about 4 mm., less closely woven than *latipes*, and with a thickened anterior margin and long anterior process.

Notes.—This species has not previously been recorded in North America. Edwards described both sexes from specimens collected in England, and has recorded it (6, p. 41 and 7, p. 244) from various localities in the British Isles, where it appears to be somewhat rare. Apparently little is known of its habits. Edwards states that, "there cannot normally be more than one brood in the year." Several pupae were found on May 8, on stones in a temporary rill (Fig. 4, B) near Carleton Place, Ont., with pupae of certain other species, Two males and one female emerged on May 13, and were placed on slides with genitalia dissected. I have not seen European specimens of the species, but have compared my material with the descriptions and illustrations of the adults published by Edwards (6, p. 41), and of the pupae by Edwards (7, p. 244) and Puri (41, p. 358).

Simulium (Eusimulium) rivuli N. SP.

Male.—Closely allied to *subexcisum* Edw., but may be separated from it by the following structural characters, and by the pupa. Palpi almost as long as antennae; hind basitarsus about 5 × 1, and four-fifths the width of tibia. *Genitalia* (Fig. 6, D): similar to *subexcisum*, but about one-fourth smaller; hairs on adminiculum longer, basal prongs not distinctly incurved; teeth on adminiculum arms larger.

Holotype.—Male, near Carleton Place, Ont., emerged from pupa 13. V. 35: Can. Nat. Coll. (slide).

Paratypes.—One male, dissected from pupa taken near Hull, Que., May 19, 1935 (slide).

Pupa.—Length 3.1 mm. Respiratory filaments about 3.6 mm., of equal thickness; four in number in two pairs: the first pair almost sessile; the second, distinctly stalked. The cocoon has a long anterior process, and is similar to the cocoon of *subexcisum* Edw.

Notes.—The male emerged on May 13, 1935, from a pupa taken, May 8, in a temporary rill, or drainage ditch, with a pebbly bottom (Fig. 4, B) several miles from Carleton Place, Ont. Unfortunately the specimen was cleared and mounted with its genitalia dissected before a note was made of its color and pubescence. Two other pupae were secured but died: one was collected with the type; the other came from S.R.S. 1, a temporary rill of similar character, near Hull, Que. In the latter, an adult male had almost completed its development, and I was able to dissect out the genitalia. The pupae were found in association with *subexcisum*, *latipes*, and several other species.

Simulium (Eusimulium) latipes MEIGEN

Simulium latipes Meigen, Klass. 1. 96. 5, 1804; Syst. Beschr. 1. 297. 11, 1818.

A pupa and cocoon that agree with the description of the European *latipes* published by Puri (41, p. 352), were collected on May 19, 1935, on a stone in a temporary rill (S.R.S. 1)* near Hull, Que., with larvae and pupae of several other species. The pupa is smaller than Puri's specimens, measuring 3.2 mm. The respiratory filaments are about 3.7 mm., four in number, arising from a short main trunk in two distinctly stalked pairs held in the vertical plane. The cocoon is closely woven, its anterior border strongly thickened and extending forward in a flat median process about 1.1 mm. long. It measures 3.6 mm., without the process, and is about one and one-quarter times as long as broad. The floor is restricted to the posterior half of the cocoon.

Simulium latipes has not previously been recorded in North America, but is widespread in Europe. Edwards (7, p. 239) states that its habitat, in the British Isles, is small temporary rills and streams, with stony bottoms, the flies emerging in spring. He recorded the females biting humans.

Simulium (Eusimulium) johannseni HART

Simulium johannseni Hart, 27th Rept. Sta. Ent. Ill. p. 32, 1912.

Dyar and Shannon (5, p. 23) record the taking of *johannseni* at Waubamic, Parry Sound, Ontario, by J. M. Aldrich, on June 8, 1915. There appear to be no other Canadian records. I have not found specimens of this species.

Simulium (Eusimulium) baffinense N. SP.

Female.—Length, 2.7 mm.; wing 3 mm. Integument black, grayish pollinose; pubescence yellow. *Head:* front rather narrow, sides moderately divergent; median furrow distinct; front and clypeus grayish pollinose, sparsely pale haired; postocular hairs largely yellow, some black. Antenna 11-jointed, entirely black, rather slender, tapering, basal joints slightly enlarged; maxillary palpi black, nearly as long as antenna. *Thorax:* mesonotum with fairly dense, sub-appressed, rather scale-like pilosity, golden on the disc, longer and paler along the margins; the integument of the mesonotum bears three faintly marked vittae, obscured by the hairs, the lateral ones diverging posteriorly; scutellum with numerous upright yellow, marginal hairs; postnotum bare, pollinose; pleural tuft yellowish. *Wings:* yellowish hyaline; anterior veins brown; posterior veins not darkened; hairs on stem vein yellow, or with a few black ones intermixed; hairs on costa intermixed with spinules, these most numerous and prominent apically; sub-costa with a weak row of hairs on basal half; radius rather sparsely haired throughout its length, those on apical half in a single row alternating with spinules; the radial sector simple and bearing a single row of fine hairs; basal cell present, minute, indistinct; halteres pale, the stem black. *Legs:* dark-brown to black, with yellowish pile; apices of femora, and bases and apices of tibiae with chitinized

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* *After careful search, I found two pupae of the same species in this stream in May, 1936; a female emerged from one on May 16, and a male from the other on June 1. The characters given in Edwards' (6 and 7) keys and description indicate them to be S. latipes Mgn.*

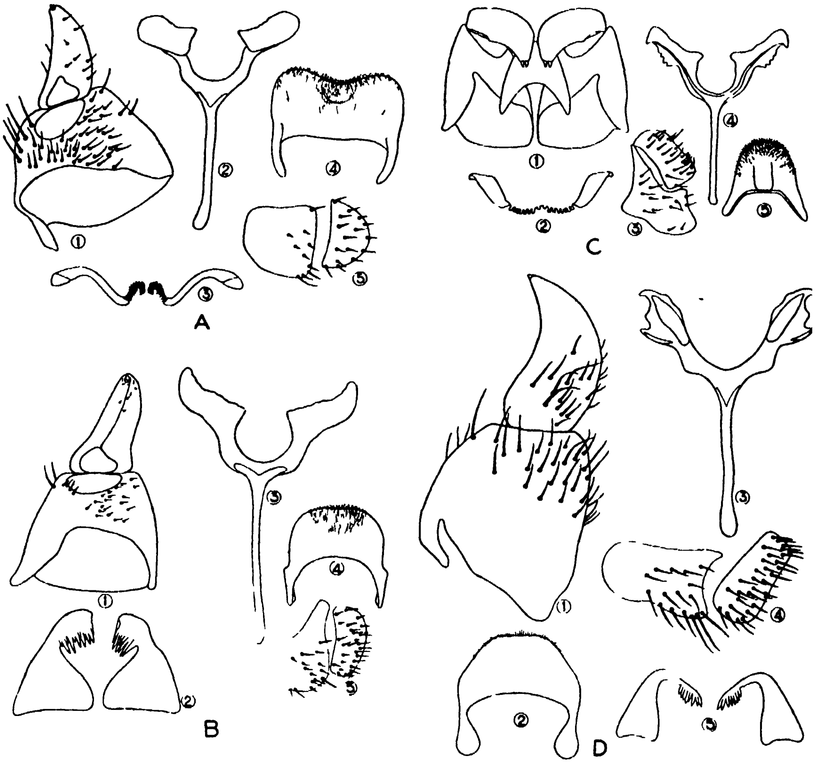

FIG. 8. *A. Parts of genitalia of Simulium (Eusimulium) baffinense n. sp.: male (1) side-piece and clasper, (4) adminiculum, (3) adminiculum arms; female (2) genital rod, (5) anal lobe and cercus. B. Simulium (Eusimulium) boreale (Malloch): male (1) side-piece and clasper, (4) adminiculum, (2) adminiculum arms; female (3) genital rod, (5) anal lobe and cercus. C. Simulium (Eusimulium) mutatum (Malloch): male (1) side-pieces and claspers, (5) adminiculum, (2) adminiculum arms; female (3) anal lobe and cercus, (4) genital rod. D. Simulium (Eusimulium) lascivum n. sp.: male (1) side-piece and clasper, (2) adminiculum, (5) adminiculum arms; female (3) genital rod, (4) anal lobe and cercus.*

black bands (very distinct in cleared specimens); basitarsus and second hind joint less heavily chitinized than front and mid tarsal joints; fore basitarsus cylindrical, about four-fifths as long as antenna; hind basitarsus about 6 × 1, and two-thirds width of tibia; calcipala small; pedisulcus present, near the base of the second hind tarsal joint, the incision deep and distinct; claws simple. *Abdomen:* basal fringe pale yellow; dorsum with somewhat sparse yellowish pubescence, hairs longer and more numerous at the sides; width of tergal plates three to seven moderately reduced; sternites two to six entirely membranous. *Genitalia* (Fig. 8, A, 2, 5): ovipositor rather short and broad; valves membranous, inner margins chitinized. Anal lobe small, chitinized, sub-quadrate, sparsely setose distally, membranous and spiculate ventrally, postero-ventral margin lobed; posterior margin above lobe with a heavily chitinized rounded protuberance ventrad and anterior to cercus, bearing several rather long setae (some of characters not well shown in figure). Arms of genital rod expanded outwardly into rounded quadrate, weakly chitinized plates.

Male.—Similar to female, but postocular hairs black; hairs on stem vein black; sub-costa bare; halteres black; legs entirely black; hind tibia and basitarsus somewhat enlarged, the basitarsus about 4 × 1, and four-fifths the width of tibia; claws with a rather strong basal tooth; abdominal tergites not greatly reduced; only the second sternite entirely membranous. *Genitalia* (Fig. 8, A, 1, 3, 4): side-piece large, conic-quadrate; clasper a little shorter, tapering, and terminating with a small tooth. Adminiculum twice as wide as long, weakly chitinized and clothed with longish hairs; upper margin depressed; basal prongs prominent, chitinous and slightly incurved. Adminiculum arms with numerous short teeth mixed with fimbriae; narrow laterally.

Holotype.—Female, Lake Harbour, Baffin Island, 10. VIII. 35 (W. J. Brown). Can. Nat. Coll.

Allotype.—Male, same data.

Paratypes.—Two females, nine males, with types. A pair of these were pinned in copula and have been cleared and mounted on the same slide.

I have also five females structurally indistinguishable from the types, but with distinctly paler pubescence; the mesonotal vittae more distinct; the hairs on radial sector arranged in an irregular double row.

In the event that these characters prove to be constant, the name ***pallens*** is proposed for this form.

Simulium (Eusimulium) boreale (Malloch)

Prosimulium borealis Malloch, Rept. Can. Arctic Exp., 1913–18, Vol. 3, p. 41 C, 1918.

Female.—Length about 2.5 mm.; wing 3.5 mm. Integument black, dusted with gray to brownish pollinosity. Differs from *baffinense* as follows: vestiture including the anterior veins of the wings, pale, silvery; pleural tuft longer, more conspicuous; a tuft of pale pile on the mesopleural membrane; mesonotal vittae more distinct; hairs on stem vein silvery; the row of hairs on sub-costa confined to outer three-fourths of vein; hairs on radius more numerous; basal cell small, but distinct; legs entirely dark; chitinous black bands absent; fore basitarsus about six-sevenths the length of antenna; hind basitarsus about 7 × 1; calcipala and pedisulcus absent; claws with a rather strong, pointed, basal tooth; reduction of width of tergal plates slightly less marked. *Genitalia* (Fig. 8, B, 3, 5): ovipositor valves short, broad at base, membranous, except along inner margins, sparsely spicular, tips not reaching ninth sternite. Anal lobe weakly chitinized, more or less triangular, ventral margin rounded; setae rather numerous on postero-ventral two-thirds. Cercus sub-quadrangular, broader than long, slightly narrowed below, setose. Arms of genital rod expanded into more or less quadrangular plates, each with a transverse, strongly chitinized ridge which forms a large blunt tooth (not shown in figure).

Male.—Postocular hairs black; general pilosity pale as in female, the hairs on the mesonotum rather sparser and longer; vittae less distinct; hind basitarsus about 4 × 1, as wide as tibia; tergites and sternites not much reduced. *Genitalia* (Fig. 8, B, 1, 2, 4): side-piece and clasper similar in size and shape to *baffinense*, but with fewer setae; adminiculum about three-fourths as wide, and basal prongs slightly produced laterally. Adminiculum arms with long, conspicuous teeth medially, fimbrious above; lateral plates broadly expanded.

Notes.—Malloch's type material was collected on the Wollaston peninsula, Victoria Island, during the summer of 1915, by D. Jenness; a female specimen was taken at Bernard Harbour, Northwest Territories, August 25, 1916, by F. Johansen (30). I have examined the genitalia of Malloch's type specimen, which is in the Canadian National Collection, and find it to be a female, not a male, as erroneously stated by him. This mistake was doubtless due to the rather poor condition of the specimen, which superficially resembles a male owing to the collapsed condition of the eyes.

Dyar and Shannon (5, p. 22) record the collection of three females by Dr. Dyar at Two Medicine River, Montana, on July 27, 1921. My material consists of a male and seven females collected at Lake Harbour, Baffin Island, August 5–10, 1935, by W. J. Brown.

Hearle (20, p. 11) states that this species is one of the commonest in the Kamloops district, British Columbia, and that he reared large series of both sexes from a number of small streams, rather slow-flowing, over rocks. There are about a dozen specimens of both sexes in the National Collection reared by Mr. Hearle in June, 1930, and May, 1931. An examination of slide preparations of the genitalia of one of each sex shows them to be identical with the specimens from Baffin Island, and with the type. Hearle described the hitherto unknown pupa and cocoon as follows: "Each breathing organ has five stout rootlike tubes of varying sizes spread fingerwise and arising from a bulbous base, two tubes paired; from all five tubes are a very large number (approximately 200 in all) of short hairlike white filaments of about equal size and mainly simple. Case pale-grey, of smooth texture and boot-shaped".

Simulium (Eusimulium) mutatum (Malloch)

Prosimulium mutatum Malloch, U.S. Dept. Agr. Bur. Ent. Tech. Ser. No. 26, 1914, p. 20.

Undescribed species No. 2, Ritcher, Ent. News XLII : 241, 1931.

Female.—Length, 2–3 mm.; wing, 2.5–3.5 mm.; integument grayish or brownish to black, sub-opaque. *Head:* front narrow, sides moderately divergent, median groove distinct; clypeus longer than broad; front and clypeus sparsely pale haired; orbital hairs largely pale. Antenna 11-jointed; entirely black; joints sub-equal except the second, which is somewhat enlarged, and the apical joint, which tapers to a point; palpi black. *Thorax:* mesonotum moderately densely clothed with grayish-yellow, hair-like pilosity; scutellum with pale and dark hairs intermixed; postnotum bare, silvery pollinose, shining; pleural tuft pale. *Wings:* hairs on stem vein black; other hairs dark, those on the apical two-thirds of costa mixed with numerous spinules; hairs on lower surface of sub-costa in a double row at the base, singly outwardly; radial vein hairy throughout its length, the distal hairs in a single row. Radial sector simple; a small basal cell present and distinct; halteres pale-brown. *Legs:* yellowish-brown to dark-brown, more or less concolorous; pilosity largely pale-yellow. Fore and mid tarsi cylindrical; hind tibial spurs unusually long, length nearly four-fifths width of tibia; calcipala distinct; pedisulcus absent; claws simple. *Abdomen:* basal fringe pale; general pubescence pale, sparse, minute, and largely confined to apical segments. Width of chitinized plates of tergites three to six markedly reduced; tergites two and seven also reduced, but less so; sternites of all but the first and terminal segments membranous. *Genitalia* (Fig. 8, C, 3, 4): ovipositor valves small, inconspicuous. Anal lobe narrowed behind cercus, expanding ventrally to about twice the length of cercus; distal portion lobed, setose, and projecting beneath it; cercus about twice as wide as long, setose. Arms of genital rod broadly and quadrately expanded; lower margins irregularly toothed.

*Male**.—Similar in size, but distinctly darker than the female. Mesonotum dull velvet-black, with rather sparse, scattered, bronzy-brown to black scale-like pilosity; marginal fringe on scutellum black; pleural tuft longer than in female, black; halteres dark-brown. Legs uniformly darker and more hirsute, the hairs bronzy black, and longer, especially on the fore femora and hind legs. Hind tibia and basitarsus broader than in female, but in the same relation to each other, the basitarsus about five-sixths the width of tibia. Calcipala smaller; hind second tarsus with an indistinct pedisulcus. Claws trifid. Abdomen velvet black, the apical margins paler; basal fringe black; pubescence short, rather sparse, bronzy black. Tergal plates less markedly reduced than in female, the reduction progressively decreasing from segments two to seven; only the sternite of second segment entirely membranous. *Genitalia* (Fig. 8, C, 1, 2, 5): side-piece conic-quadrate, longer than broad, setose distally. Clasper setose, shorter than side-piece, rather wide, uniform, but suddenly tapering near tip and bearing a pair of closely set, blunt, terminal teeth. Adminiculum broadly rounded, chitinized, hirsute; basal prongs strong, divergent. The adminiculum arms bear a marginal row of irregular, blunt teeth medially.

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* ***Previously undescribed.***

Allotype.—Male, near Carleton Place, Ont., reared from pupa, 9.V.35. Can. Nat. Coll.

Paratypes.—Three males, same data, 9–13. V. 35.

Pupa.—Length about 3–4 mm.; respiratory tuft about three-quarters the length of pupa. The main trunk divides close to the base into two branches held in the vertical plane. The upper branch further subdivides into seven filaments; the lower, into five, forming twelve in all. In some specimens the unpaired filament of the lower group divides into two near the apex, or one of the paired filaments gives rise to a short branch, or a weak filament arises from the main stem of the upper group. Thus the number of filaments varies from twelve to fourteen, but the usual number is thirteen. The cocoon is similar to that of *Simulium (Prosimulium) gibsoni;* indefinite in shape and consisting of loosely woven silk encrusted with coarse sand particles.

Synonomy.—Under the name *Undescribed Species No. 2*, Ritcher (44, p. 241) published a description of the immature stages of a species collected from a small temporary woodland stream near Urbana, Illinois, in April, 1930. Comparison of his descriptions and figures of the larva and pupa with my specimens show them to be identical.

Notes.—During May, 1935, I found small numbers of larvae and pupae in three small temporary streams flowing through rough pasture land in the Ottawa district, and succeeded in rearing four males and eight females. These males are the first to be recorded, and this is the first time that the immature stages have been associated with the adults. The species was found in association with *venustum*, *latipes*, *subexcisum*, *decemarticulatum* and *gibsoni*. The details of the collections are as follows. On May 8, larvae and pupae were found on stones in a shallow drainage ditch (C.P.H. 1), and in a rock-bottomed stream (C.P.H. 2), several miles from Carleton Place, Ont. (Fig. 4, A and B). Three males emerged from the pupae on May 10, and a male and a female on May 13. Several larvae and pupae were also collected in a somewhat grass-grown, pebbly-bottomed rill (S.R.S. 1), near Hull, Que., on May 19. From these, seven females emerged on May 21, and 23. The temperature of the three streams ranged from 55° to 62° F.

Distribution.—*S. mutatum* has a wide distribution in North America. Malloch (29, p. 20) described the species from a female taken at Glassboro, New Jersey. He also recorded it from localities in Illinois, Missouri, Washington, British Columbia and Alaska. Dyar and Shannon (5, p. 17) added Virginia, Maryland and Indiana. I have specimens from the following localities. *Quebec:* Hemmingford, May 15 and July 25, 1928 (G. H. Hammond); Montebello, May 21, 1930 (N. J. Atkinson); Pink's Lake, near Hull, June 5, 1931 (C. R. Twinn), also reared females from near Hull, May 21 and 23, 1935. *Ontario:* Niagara Glen, June 23, 1926 (G. S. Walley); near Carleton Place, reared males and females, May 10 and 13, 1935 (C. R. Twinn).

Simulium (Eusimulium) lascivum N. SP.

Female.—Length, 3–4 mm.; wing, the same; integument dark-brown to black, with pollinose dusting. *Head:* front moderate, about twice as wide at vertex as at base of antennae; median furrow distinct; clypeus somewhat broader than long; front and clypeus black, covered with dull yellowish pollinosity, and short decumbent yellowish pubescence; orbital hairs black. Antenna 11-jointed, joints four to ten sub-equal, the three basal joints larger, and the apical joint more elongate, tapering to a rounded point; color dull-black, the second joint and the distal margin of the basal joint reddish; palpi black. *Thorax:* mesonotum black with a faint yellowish or grayish pollinose dusting, and fine short yellow pubescence; three rather indistinct mesonotal vittae present, the median stripe a thin straight line, lateral ones broader, somewhat sinuate. Scutellum dark-brown to black with sparse yellow pubescence and rather short black hairs; postnotum black, pollinose. Pleurae dark-brown to black posteriorly; the lower part black with whitish pollinosity; pleural tuft small, black, with a tinge of yellow. *Wings:* hairs on stem vein black; costa with numerous short black hairs mixed with minute spinules; sub-costa with a single row of hairs; radial sector shortly and indistinctly furcate, the posterior branch with a single row of hairs on the upper surface; small basal cell present; halteres light-brown. *Legs:* coxae and underside of fore trochanters black, whitish pollinose; other joints dark-brown, with fine black and yellowish pilosity; hind tibial spurs prominent; hind basitarsus about 6 × 1, and five-sevenths width of tibia: calcipala minute; length of second hind tarsus one and one-half times the width of basitarsus; pedisulcus present but shallow; claws long, slender, with a minute sub-basal tooth. *Abdomen:* black; grayish pollinose; apices of all tergites pale-brown, terminal segment almost wholly brown; basal fringe short, of fine yellowish hair; pubescence on dorsum sparse, minute, yellowish; longer and more numerous black and yellow hairs on lateral margins of dorsal plates, and on sternal plates; tergites well developed and chitinized, none greatly reduced in width; ninth tergite (viewed laterally) dorsally emarginate and caudally prolonged, the prolongation narrow, setose; sternites one and two membranous. *Genitalia* (Fig. 8, D, 3, 4): ovipositor valves as broad at base as long, tapering, extending to one-third length of ninth sternite; weakly chitinized, spiculate, inner margins strongly chitinized; tips divergent. Anal lobe quadrangular, longer than wide, length slightly exceeding width of cercus; more heavily chitinized postero-ventrally; slightly prolonged beneath cercus; strongly setose except anteriorly and apically. Cercus quadrangular, twice as wide as long, setose. Arms of genital rod widely divaricate, expanded, toothed, the expansions each reinforced with a chitinous bar.

Male.—Essentially similar to female, but smaller; antenna entirely black; hind tibia about one-fifth wider than basitarsus, both much swollen; hind basitarsus about 3.5 × 1; length of second hind tarsal joint slightly less than width of basitarsus; basal tooth on claws more prominent. *Genitalia* (Fig. 8, D, 1, 2, 5): side-piece and clasper unusually large; side-piece conical, with long setae distally; clasper almost as long as side-piece, with long setae basally and tapered, curved, terminating with a small tooth. Adminiculum similar to *mutatum*, but about twice as wide, and the basal prongs roundly expanded. Adminiculum arms with a row of small, pointed, marginal teeth.

Holotype.—Female, Rockcliffe, Ont., reared from pupa, 13. V. 35; Can. Nat. Coll.

Allotype.—Male; same data.

Paratypes.—Four males, 5 females, Rockcliffe, Ont., emerged 13. V. 35; 6 males, 7 females, Carleton Place, Ont., emerged 27. V. 35.

Pupa (Plate I, Fig. 4).—Length 4–6 mm.; respiratory tuft bush-like in shape, usually less than one-half the length of the pupa, and composed of about 30 slender filaments (counts of twelve specimens showed as few as 27 and, in one case, as many as 40; the average was 30) arranged in a number of groups branching from a short, stout, base. The cocoon is of the *Prosimulium* type, consisting of a loosely woven silk sheath, indefinite in shape.

*Notes**.—Larvae and pupae were found in great numbers in May, 1935, in a small stream at Rockcliffe, Ont., connecting McKay Lake with the Ottawa River, and also in the falls and rapids of the Mississippi River, at Carleton Place, Ont. I first collected pupae in the stream at Rockcliffe, on May 29, 1928, but did not then rear adults.

The McKay Lake stream (McL.) is about half a mile in length, and rarely exceeds several feet in width and a few inches in depth. During the summer, with the fall of the lake level, it often dries up. The bed is of clay formation, and near the source of the stream there is a heavy growth of bulrushes and grasses. When first examined on April 13, 1935, the temperature of the water was 41° F., and only a few mature overwintering larvae of another species were found. On April 26, when the temperature of the water was 56° F., immense numbers of young larvae were present on the dead grasses, leaves and other obstructions in the water, the concentration being heaviest near the source of the stream. It seems probable that they had recently hatched from eggs laid the previous season. The young larvae were pale reddish-brown in color, before immersion in alcohol, owing to patches of pigmented cells in the thorax and abdomen. The intestinal tracts showed green through the integument, indicating that the larvae were feeding on the algae which occurred in gelatinous masses on the grasses covering the stream bottom. By May 9, when the water temperature was 59° F., the majority were mature and many had pupated. The infestation had become more

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* *A collection of males received in July, 1936, from Dr. Robt. Matheson, of Cornell University, taken at Cayuta Lake, N.Y., on May 21, 1935, proved to be lascivum.*

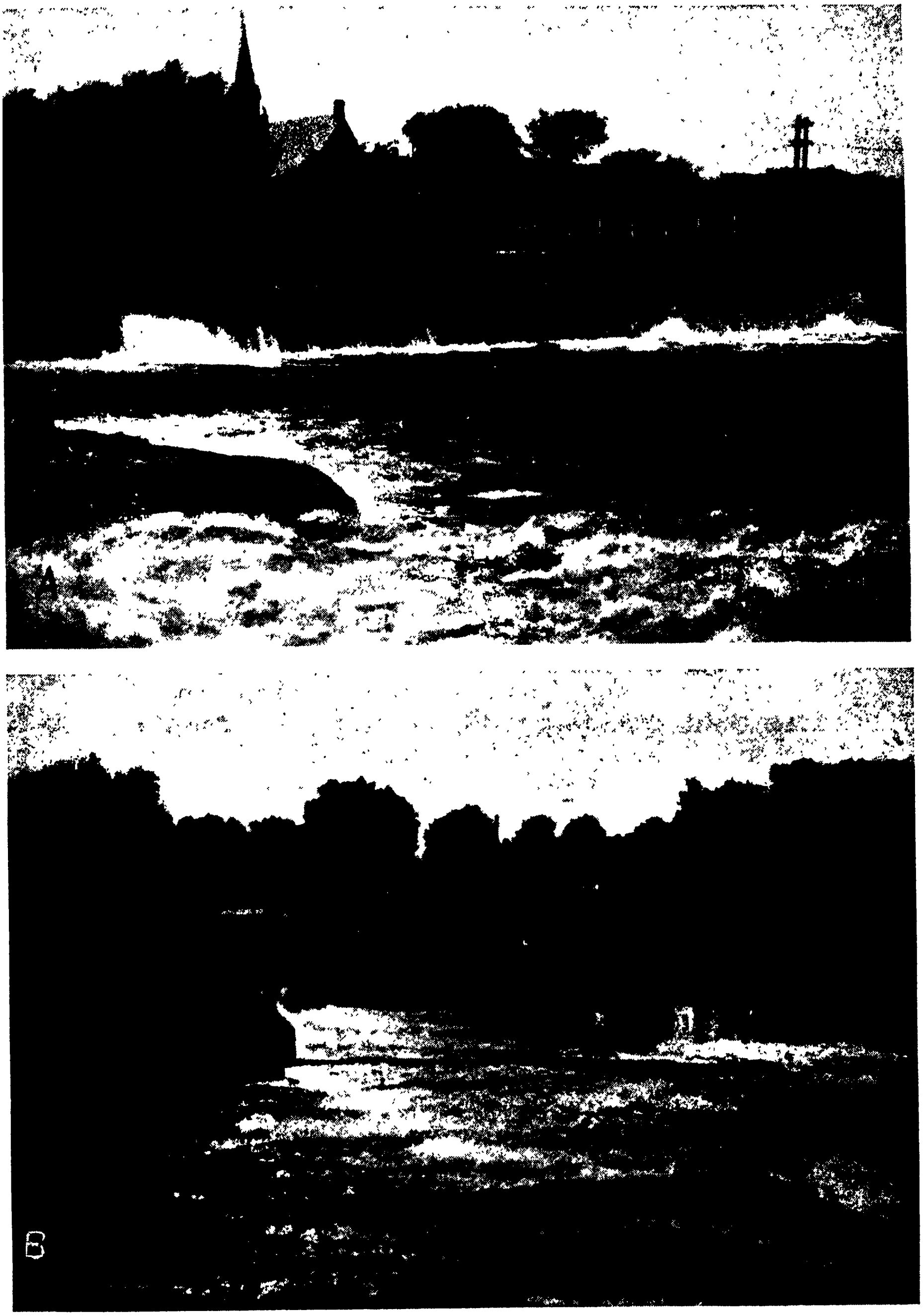

FIG. 9. *A. Mississippi river, Carleton Place, Ont.; breeding place of S. lascivum n. sp., S. pictipes Hgn., and other species. B. Another view of the above; S. ottawaense n. sp., was found to the right.*

evenly distributed throughout the course of the stream. Stones, rocks and other obstructions were thickly covered with the pupae which were hidden from casual observation by the gray-green webbing of the cocoons, and often by writhing masses of larvae. Adult flies began to emerge a few days later. By June 8, the stream was reduced to a tiny rill, and only the immature stages of *vittatum* could be found.

The Mississippi river (Fig. 9, A and B) issues from the lake of the same name above Carleton Place, Ont. Where it passes through the town it is rather shallow and swift and has a bottom of shaly rock, its passage interrupted by concrete dams. During the spring freshet the river level is high and the current strong, but often during the summer it is much diminished, and in places almost dry.

When visited on May 8, 1935, the river was high and flowing over the dam with a roar. The temperature of the water was 53° F. Examination revealed the presence of myriads of larvae on the dam oscillating in dark-green, moss-like masses in the current. The larvae were small to well-grown, but none showed the developed pupal respiratory histoblasts. When visited again on May 24, many had pupated, the pupae inconspicuous under the protective grayish covering of the cocoons. The temperature of the water was 58° F. Emergence of the flies had commenced, and numerous males were flying erratically above the water surface of the falls, or resting on the retaining walls nearby. A collection of the pupae was made and a goodly number of both sexes emerged in confinement, on May 27. It is evident that the species reaches the peak of its emergence from the Mississippi river during the latter part of May, as no trace of it was found during subsequent months either in 1935, or in previous years. On May 30, 1933, I found the adults crawling in thousands on nearby walls, many of them mating.

S. lascivum was the only species observed to mate in captivity. Numerous adults of both sexes that emerged on May 13, were actively mating in the small, damp, rearing vials, at 8 a.m. During copulation the male bends the tip of his abdomen downward and forward to connect with that of the female. Several specimens frequently clung together during this operation, tumbling about awkwardly within the confining walls of the vial. It was observed, too, that *lascivum* is not strongly attracted to light as are the adults of the other species reared.

(To be concluded in next issue.)

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THE BLACKFLIES OF EASTERN CANADA (SIMULIIDAE, DIPTERA).[1] PART II.

BY C. R. TWINN

Subgenus *Simulium* Latreille

Simulium Latreille, Hist. Nat. Ins. et. Crust., 3 : 426, 1802.
Atratocera Meigen, Klass., 1 : 94, 1804.
Boophthora Enderlein, D. Tier. Woch., 29 : 199, 1921.
Chirostilbia Enderlein, ibid.
Edwardsellum Enderlein, ibid.
Gynonychodon Enderlein, ibid.
Odagmia Enderlein, ibid.
Wilhelmia Enderlein, ibid.
Gomphostilbia Enderlein, ibid.
Stilboplax Enderlein, ibid.
Friesia Enderlein, Konowia, 1 : 69, 1922.
Discosphyria Enderlein, ibid., 1 : 72.
Byssoden Enderlein, Zool. Anz., 62 : 209, 1925.
Pseudosimulium Baranoff, Neue Beitr. Sys. Ins., 3 : 164, 1926.
Pseudodagmia Baranoff, ibid.
Pternaspatha Enderlein, Arch. Klass. phyl. Ent. 1 : 88, 1930.
Anasolen Enderlein, ibid., 1 : 94.
Notolepria Enderlein, ibid., 1 : 95.
GENOTYPE.—(*Culex colombaschensis* FABRICIUS) = *Culex reptans* LINNAEUS.

KEY TO THE FEMALES OF *Simulium*

(Based on external characters and genitalia)

1. Fore coxae black, grayish pollinose; mesonotum distinctly striped; abdomen opaque-gray with black markings 2
   Fore coxae yellow; mesonotum not distinctly striped; abdomen dark-brown or black 3
2. With three mesonotal vittae; hairs on stem vein black; sub-costa with a ventral row of hairs . . . . *Simulium pictipes* Hgn.
   With five mesonotal vittae; hairs on stem vein pale; sub costa bare . . . . *Simulium vittatum* Zett.
3. Pleural tuft pale 4
   Pleural tuft black 6
4. Claws with a small sub-basal tooth; medium sized species close to *venustum;* anal lobe rounded quadrate, as long as ninth tergite, moderately setose posteriorly . *corbis* n. sp.
   Claws simple 5

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[1] *Concluded from September issue.*

5. Tergites six to nine black, shining; anal lobe strap-shaped, shorter than cercus (horizontally), and less than twice as wide, evenly setose; hairs on stem vein all pale . . . . *venustum* Say
   Tergites six to nine grayish black, sub-opaque; anal lobe pyriform, narrow behind cercus, broadly rounded below, twice as long as cercus and twice as wide, anterior margin bare of setae; hairs on stem vein variable, pale and dark intermixed . . . . *ottawaense* n. sp.
6. Sub-costa with a row of hairs on ventral surface; anal lobe not produced anteriorly; about same horizontal length as cercus; medium sized species . . *perissum* D. & S.
   Sub-costa bare; median area of anal lobe produced anteriorly to about twice length of cercus; smaller species 7
7. Forks of genital rod tapered beyond tooth; hairs on stem vein all black . *fibrinflatum* n. sp.
   Forks of genital rod roundly expanded beyond tooth; hairs on stem vein variable, yellow, bronzy-brown, black, or the pale and dark hairs mixed . *nigroparvum* n.sp.

KEY TO THE MALES OF *Simulium*

(Based on the genitalia)

1. Clasper with three terminal teeth or none . . . . , 2
   Clasper with a single terminal tooth, or spine 3
2. Clasper shorter than side-piece, with three terminal teeth; adminiculum arched . . . . *vittatum* Zett.
   Clasper longer than side-piece, terminal teeth absent; adminiculum deeply cleft mesially . . . . *pictipes* Hgn.
3. Adminiculum narrow, inverted Y-shaped, with posterior process or keel . . . 4
   Adminiculum broader, tooth-shaped, without posterior process 5
4. Posterior process apically rounded, marginally denticulate . . . . *corbis* n. sp.
   Posterior process rounded, smooth, setulose, without marginal denticles . *ottawaense* n. sp.
5. Clasper with a small inner basal process; prongs of adminiculum simple . . . 6
   Clasper simple; prongs of adminiculum each with a posterior sub-basal arm . . 7
6. Basal process of clasper smooth; side-piece with rather numerous setae; adminiculum quadrate . . . . *venustum* Say
   Basal process spinose; side-piece setose only on distal margin; adminiculum subquadrate, apically emarginate and denticulate . . . . . . *perissum* D. & S.
7. Adminiculum one and one-fourth times as long as broad, upper margin denticulate at sides, hirsute medially . . . . *fibrinflatum* n. sp.
   Adminiculum twice as long as broad, upper margin denticulate throughout, hairs sparse or absent . . . . *nigroparvum* n. sp.

Simulium (Simulium) vittatum ZETTERSTEDT

Simulium tribulatum Lugger, 2nd. Rep. Ent. Minn. p. 205, 1896.

Simulium glaucum Coquillett, Proc. U.S. Nat. Mus., Vol. 25 : 97, 1903.

Simulium dahlgruni Enderlein, Deut. Tierarz. Woch. Hanover, 1921, p. 43; zool. Anz. Vol. 53, p. 45.

Female.—Length, 2–3 mm.; wing, 2.8–4 mm. Integument black, covered with opaque gray pollinosity, and brown to black markings; pubescence pale, rather sparse. *Head:* opaque-gray; front broad, with sparse pale hairs; clypeus as broad as long, about as wide as front at vertex, hairs longer, more numerous. Antenna 11-jointed, the two basal joints brownish, remainder black, tapering; palpi black. *Thorax:* mesonotum with five dark-brown to black vittae; pubescence short, sparse, pale; scutellum with a few recumbent pale scales and numerous upright pale hairs; postnotum silvery pollinose with a central dark spot; pleural tuft pale. *Wings:* hairs on stem vein and base of costa pale, remainder black; those on costa and a row on the outer part of radial vein mixed with spinules; sub-costa bare; radius bare between stem vein and base of radial sector; sector simple, reaching the costa close to the tip of radial vein, lower surface with a row of hairs; basal cell absent; halteres pale, yellowish, the base of stem black. *Legs:* black, except the basal half of tibiae and mid and hind basitarsi which are yellowish; fore coxae gray pollinose; fore tibiae with a basal pollinose white patch; fore tarsi slightly flattened; hind basitarsus about 6 × 1, and two-thirds the width of tibia; calcipala small; pedisulcus distinct; claws simple. *Abdomen:* opaque-gray, with three rather indefinite rows of opaque black spots on the dorsum, the median row often coalescing to form a central black stripe; basal fringe and general, short, sparse pubescence, pale; chitinization of tergites three to five reduced to small median plates; sternites two to seven entirely membranous. *Genitalia* (Fig. 10, 4, 5): valves of ovipositor membranous. Anal lobe rather sparsely setose, about as long as cercus, but twice as wide (ventral measurement), one-half its width conically lobed ventrad of cercus and concealing the ovipositor; lower margin clear and setulose. Cercus rounded distally; setose. Arms of genital rod quadrately expanded, the corners produced; chitinization of outer portion denser than inner.

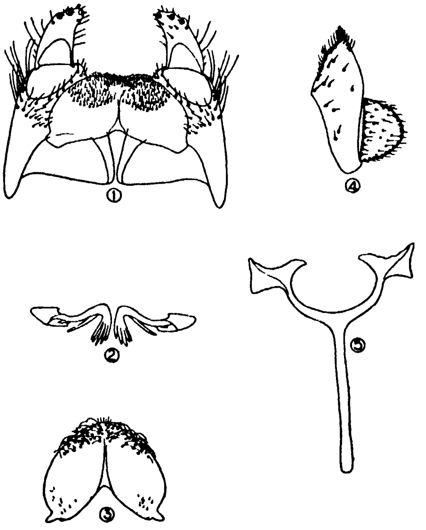

FIG. 10. *Parts of genitalia of Simulium (Simulium) vittatum Zett.: male (1) side-pieces, claspers and adminiculum, (2) adminiculum arms, (3) adminiculum detached; female (4) anal lobe and cercus (ventral side up), (5) genital rod.*

Male.—Distinctly darker than female. Antenna entirely black, more slender. Mesonotum white or silvery pollinose on lateral and posterior margins; disk velvety black and bearing two short, rather indefinite pale stripes; pilosity hair-like, moderate, yellowish. Hairs on stem vein and base of costa black. Legs as in female, but darker, the hairs longer, especially on the fore femora and posterior margins of the hind legs. Abdomen opaque black with silvery pollinose patches on the sides; basal fringe dark-brown to black, other pilosity pale, rather long; none of the tergal plates greatly reduced; only the second sternite entirely membranous. *Genitalia* (Fig. 10, 1-3): side-piece longer than wide, conical; clasper shorter than side-piece, obliquely truncate, with three terminal teeth. Adminiculum broad, convex above, emarginate below; rather weakly chitinized, hirsute, basal prongs abbreviated. Adminiculum arms chitinous, laterally expanded; inner margin with elongate teeth.

Pupa (Plate I, Fig. 6).—Length 3–5 mm.; the respiratory filaments about three-fourths that of the pupa. The respiratory tuft consists of a short main trunk which gives rise to eight branches, each again dividing to produce sixteen filaments in all. The cocoon is of the usual wall-pocket type.

Notes.—This blackfly is one of the most common species in the Ottawa district, both in Ontario and Quebec, and occurs in many of the smaller rivers and streams in that area, often in great abundance, and usually associated with *Simulium venustum.* It attacks domestic animals, particularly horses, and occasionally man. There are two or three generations a year, the broods overlapping, so that during the summer months all stages may commonly be found together. I have collected pupae as early as April 12, and as late as the end of September. The winter is spent in the larval stage. Larvae taken from a small stream by breaking through the covering of ice and snow, on Dec. 15, 1931, were mostly well grown, although some were barely one-third grown. In all of them the histoblasts were undeveloped. Despite the low temperature they were feeding, as was evidenced by an examination of the gut contents.

The eggs and mature larvae of *vittatum* are shown on Plate I, Figs. 1 and 3. The pupae and cocoons are depicted on Plate I, Fig. 6 (opposite page 100).

Distribution.—*S. vittatum* is extremely widely distributed over North America, from Mexico to the Arctic, and from the Atlantic to the Pacific. It is a common species in Europe where it was originally described, probably from Lapland; it also occurs in Greenland. I have found the immature stages in most of the rivers and streams in the Ottawa district, wherever blackflies develop, and have reared adults of both sexes from pupae taken from streams in the wooded Gatineau hills, in Quebec, and in the more level country in Ontario, south of the Ottawa river. In addition, there are specimens in the National Collection from the following localities in Ontario: Nipigon, 1. VII. 23 (M. K. Bigelow); Biscotasing, 9. VII. 30 (K. E. Schedl); Dundonald ("in horse's ear") 27. VIII. 00; Trenton, 20. VI. 01 (Evans); Strathroy (attacking horses and cows), 22. VIII. 32, (H. F. Hudson); Toronto, 27. VII. 28 (C. R. Twinn); Algonquin Park, 18. V. 32 (C. R. Twinn). W. J. Brown collected seven females at Lake Harbour, Baffin island, August 9–14, 1935.

Simulium (Simulium) pictipes HAGEN

Simulium pictipes Hagen, Proc. Bos. Soc. Nat. Hist., 20 : 305, 1879.

Simulium innoxium Comstock, Manual for the Study of Insects, p. 452, 1895.

Female.—Closely allied to *vittatum* Zetterstedt, from which it may be separated by the following characters: Length, 3–4 mm.; thorax with three mesonotal vittae; upright hairs on the scutellum and the hairs on the stem vein of the wing black; lower surface of the sub-costa with a row of fine hairs; abdomen darker, the opaque black markings on the dorsum undivided. *Genitalia* (Fig. 11, A, 4, 5): ovipositor valves narrow, thin, membranous. Anal lobe scarcely larger than cercus, rounded quadrate, broadly conical ventrally; chitinized, especially along anterior margin, a group of spicules anterior to this; ventral and posterior margins setulose. Cercus rounded quadrangular, wider than long, setose. Arms of genital rod triangularly divaricate, the apical expanded portions chitinized and singly toothed.

Male.—Similar to male of *vittatum* Zett., but larger; two basal joints of antenna brownish; palpi about one-third longer than antenna; legs generally darker, base of hind tibiae not broadly pale; abdominal basal fringe paler, yellowish-brown; sub-costa bare. *Genitalia* (Fig. 11, A, 1-3): side-piece quadrate, outer apical angle produced; clasper longer than side-piece, rounded and somewhat constricted at middle, without terminal tooth. Adminiculum weakly chitinized, nearly twice as wide as long, broadly and deeply cleft mesially, minutely hirsute; basal prongs more strongly chitinized, tapering. Adminiculum arms with a few long, pointed teeth; lateral plates broad.

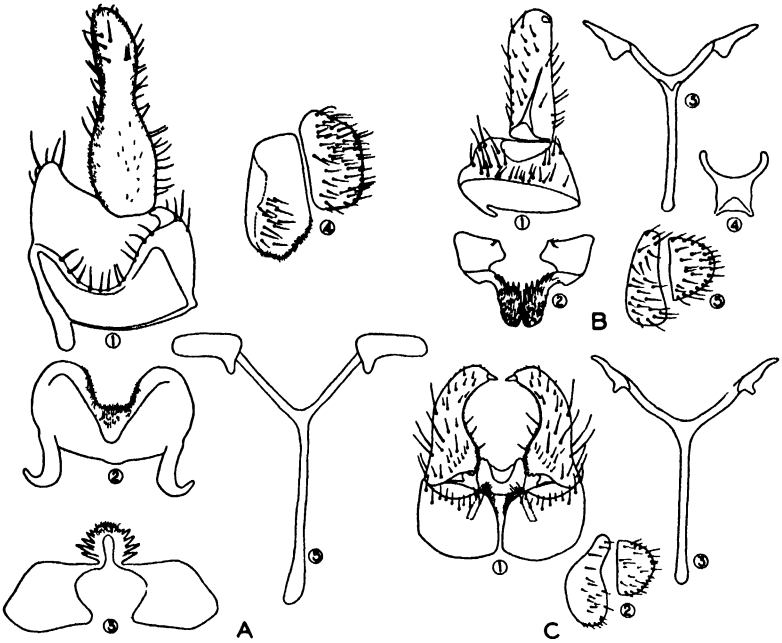

FIG. 11. *A. Parts of genitalia of Simulium (Simulium) pictipes Hagen: male (1) side-piece and clasper, (2) adminiculum, (3) adminiculum arms; female (4) anal lobe and cercus, (5) genital rod. B. Parts of genitalia of Simulium (Simulium) venustum Say: male (1) side-piece and clasper, (4) adminiculum, (2) adminiculum arms (4 and 2 ventral side up); female (3) genital rod, (5) anal lobe and cercus. C. Parts of genitalia of Simulium (Simulium) perissum D. & S.: male (1) side-pieces, claspers and adminiculum; female (2) anal lobe and cercus, (3) genital rod.*

Pupa.—Length 3.5–5 mm. The respiratory tuft is about two-fifths as long as the pupa, and consists of nine rather stout filaments, eight of them arranged in pairs on very short stalks and the ninth arising directly from the short main trunk. The cocoons are boot-shaped, with open weave, and the anterior margin not thickened. The pupa and its respiratory filaments lie concealed within the cocoon.

Notes.—I have found the immature stages of this species only in very swift water such as in, or just below, waterfalls. Masses of the pupae and large numbers of the distinctive black larvae, in various stages, were discovered under the cascading water on the steep rock face of a waterfall of the Blanche river (Fig. 7, B), at Perkins Mills, Que., on August 24, 1932. Egg masses were also plentiful in the shallow water along the sides of the falls in places where the rock was continually wetted by the spray, or by the intermittent rise and fall of the water level. Adults were flying above the falls; specimens captured were all males. Again, on July 26, 1935 (9–10 a.m.), large numbers of males were seen and many netted at the foot of another waterfall, several miles south of Perkins Mills (Fig. 12, A). These were in erratic flight from just above the surface of the swift and broken water to a height of probably 15–20 feet.

Adults of both sexes were reared on July 26, 1935, from pupae taken with difficulty from the smooth rock surface beneath the tumultuous waters of the falls of the Blanche river, at Perkins Mills; also from pupae found on August 2, 1935, in crevices in the masonry near the foot of a concrete dam in the Mississippi river, at Carleton Place, Ont. (Fig. 9, B).

Smart (48, p. 62) who studied the biology of this species at Ithaca, N.Y., found that it passes through four (possibly five) generations in a year, the winter being passed in the larval stage. The number of generations annually is probably fewer in our more northern latitude. Malloch (29, p. 57) states that the female will bite horses, and Jobbins-Pomeroy (38, p. 6) records the removal of an engorged female from a mule's ear. Smart induced specimens in captivity to bite his arm. Apparently, however, it does not normally attack humans.

Distribution.—It has been recorded hitherto only from the Eastern United States. Malloch (29, p. 56) and Dyar and Shannon (5, p. 28) list records from New York, Maryland, District of Columbia, Virginia, South Carolina, Indiana and Minnesota. My records are all from the Ottawa district including rearings and collections at Carleton Place, Ont., and Perkins Mills, Que. There are two specimens in the National Collection labelled Ottawa, 30. VII. 12 (Beaulieu) and 7. VIII. 23 (C. H. Curran). Its distribution in Eastern Canada is probably much wider than is indicated by these records.

Simulium (Simulium) venustum SAY

Simulium venustum Say, Journ. Acad. Sci. Phila. 3 : 28, 1823.
Simulium molestum Harris, Ins. Injur. to Veg., ed. 3 : 601, 1862.
Simulium piscicidium Riley, Amer. Ent. 2 : 367, 1870.
Simulium irritatum Lugger, 2nd. Rep. Ent. Minn. p. 177, 1896.
Simulium minutum Lugger, ibid.
Simulium jenningsi Malloch (part), U.S. Dept. Agr. Bur. Ent. Tech. Ser. No. 26, p. 41, 1914.
Simulium austeni Edwards, Bull. Ent. Res. 6 : 33, 1915.
Simulium rileyana Enderlein, Konowia, 1 : 75, 1922.

Female.—Length, 2–2.5 mm.; wing, 2.5–3 mm. *Head:* black; front glossy black with slight white pollinosity, as broad at vertex as clypeus; clypeus opaque white pollinose, as broad as long; antenna 11-jointed, black, basal joints reddish-brown; palpi about as long as antennae. *Thorax:* shining black, the mesonotum with whitish to brownish pollinosity, strongest at the margins, also on the pleurae; pubescence short, fine, yellowish; scutellum dark-brown, with a few recumbent pale scales and numerous upright brown to black hairs; postnotum bare, shining pollinose, a narrow silvery band along the anterior margin; pleural tuft pale. *Wings:* hairs on the stem vein and base of costa pale; remainder pale to dark, those on costa and a row on the outer part of radial vein mixed with spinules; sub-costa with a row of hairs on lower surface, terminating before apex; radius bare between stem vein and base of radial sector; the sector simple, reaching the costa close to tip of radial vein, lower surface with a row of hairs; basal cell absent; halteres pale yellowish, the base of stem black. *Legs:* largely yellowish with darker areas, notably the mid and hind coxae, the tibiae apically, the fore tarsi and terminal joints of mid and hind tarsi; all tibiae silvered on dorsal surfaces; pilosity pale to dark, moderate; fore tarsi flattened; hind basitarsus approximately 6 × 1 and two-thirds width of tibia; calcipala and pedisulcus present; claws simple. *Abdomen:* basal fringe pale yellow, general pubescence short, sparse, brown; segments three to five opaque velvet black; second segment silvery at the sides; remaining segments dark-brown to black, shining; tergal plates of segments three to five greatly reduced in width; sternites of all but the terminal segments membranous. *Genitalia* (Fig. 11, B, 3, 5): ovipositor valves small, broader than long, truncate, minutely setulose, and with a group of setae; membranous, weakly chitinized medially. Anal lobe dorsally one-half as long as cercus, increasing in length ventrally and truncately produced; setose throughout. Cercus rounded distally; evenly setose. Arms of genital rod divaricate, the more thickly chitinized expanded portions each with a broad, blunt tooth, and irregular ventral margin.

Male.—Mesonotum opaque black, with a posterior and lateral marginal band of light-reflecting silver-gray pollinosity, most conspicuous anteriorly where it forms two backward and inwardly directed silvery stripes which stop short of the middle of the disk; pubescence yellowish-brown; pleural tuft black. Hairs on stem vein and other hairs black; sub-costa bare. Legs black; basal half of anterior tibiae yellowish, dorsal surface with a white pollinose patch; mid and hind tibiae yellow narrowly at base, the dorsal surfaces faintly white pollinose and pale haired; fore coxae, base of mid basitarsus and bases of hind basitarsus and second joint, yellow; fore coxae with white dusting; hind tibia and basitarsus slightly inflated; basitarsus about 5 × 1 and two-thirds width of tibia. Abdomen opaque black, with more or less conspicuous ventro-lateral pearlaceous pollinose markings on segments two, six and seven; basal fringe and other pubescence dark-brown to black; tergal plates not greatly reduced; second sternite membranous, others with small chitinized plates. *Genitalia* (Fig. 11, B, 1, 2, 4): side-piece rounded quadrate, wider than long, setae rather numerous. Clasper about twice as long as side-piece; stout, rounded, slightly constricted beyond the middle, a small basal projection on inner side, a spine at tip. Adminiculum stout, quadrate, strongly chitinized; basal prongs divaricate, incurved near tips. Adminiculum arms with numerous long teeth mixed with fimbriae; widening, angled, and broad at base.

Pupa.—Variable in size; respiratory tuft about one-half as long as the pupa, and consisting of six filaments arranged in three pairs on short stalks held in the vertical plane, the stalks sub-equal and somewhat variable in length. The cocoon is of the usual wall-pocket type, closely woven, with a thickened anterior margin (Plate I, Fig. 5). The pupa and other immature stages have been described and figured by several authors including Malloch (29), Jobbins-Pomeroy (38), Edwards (7), Puri (41), and Johannsen (23, 24).

Notes.—*S. venustum* is probably the most common and abundant species in the Ottawa district, on both sides of the Ottawa river, in Ontario and Quebec. It is found in all types of bodies of running water, permanent and temporary; in rivers, streams, rills and drainage ditches. Pictures of such breeding places are shown in Figs. 2, 3, 4, and 7, A. The immature stages commonly occur in association with other species attached to rocks, stones, aquatic plants, logs, debris, and other obstructions in the water. The larvae are often found in remarkable numbers: 734 in various stages of development were counted on an area of approximately one square inch on a submerged tree branch in Cranberry creek, Algonquin Park, on May 18, 1932; another count on a similar area revealed 742. The adult flies are found on the wing from May to October, but occur in greatest numbers in June and early July. There are probably three or four generations of the flies in a year; the various generations overlap, and during the summer all stages may be found together. The winter is passed in the larval stage. The eggs are illustrated on Plate I, Fig. 2, and the cocoons and pupal skins on Plate I, Fig. 5. This species is a troublesome pest of animals and man, and serves as the intermediate host of a protozoon blood parasite of ducks, *Leucocytozoon anatis* Wickware, as recorded by O'Roke (34). O'Roke found that this parasite caused a mortality rate of 10–100% in ducklings, but for adult ducks less than 1%. The disease is common in Canada as I have recorded elsewhere (55).

Distribution.—*S. venustum* occurs in Europe and throughout North America. It has been taken in many localities in every province in Canada. A detailed account of its distribution in the Dominion was published in 1933 (55), and need not be repeated here.

Simulium (Simulium) perissum DYAR AND SHANNON

Simulium perissum Dyar and Shannon, Proc. U.S. Nat. Mus., Vol. 69, Art. 10, p. 43, 1927.

Simulium jenningsi Malloch (part), U.S. Dept. Agr. Bur. Ent. Tech. Ser. No. 26, p. 42, 1914.

Dyar and Shannon confined their description of *perissum* almost entirely to the genitalia of both sexes. Among adults of *venustum* reared from pupae collected in the Jock river, near Ottawa, Ont., on May 12, 1935, I found a male and female with genitalia which appear identical with those described and figured for *perissum*. The only external characters mentioned by Dyar and Shannon are contained in the statement quoted as follows: "A medium sized species, very close to *venustum* and very difficult to separate from the typical form. It is somewhat darker, with evidence of three mesonotal vittae; the stem vein is black pilose." I can find no trace of mesonotal vittae in my specimens. They differ from *venustum* as follows.

Female.—Somewhat darker; the antennae black, with basal joints faintly reddish; front sharply narrowed from vertex to base of antennae, sides strongly divergent; pleural tuft black; hairs on stem vein and base of costa black. *Genitalia* (Fig. 11, C, 2, 3): ovipositor valves broader than long, remote at tips; membranous, weakly chitinized along inner margins; minutely setulose, with one or two fine setae basally. Anal lobe with rather broad median area; moderately and irregularly setose. Cercus distally rounded; evenly setose. Arms of genital rod divaricate; the thickly chitinized part of outward expansions with a long basal tooth and irregular margin.

Male.—Generally darker in appearance than *venustum;* pollinose areas less conspicuous; legs almost entirely black, the pale portions of the hind tarsal joints indistinct; hind legs much inflated, the hind basitarsus slightly more than 3 × 1, and three-fourths the width of tibia. *Genitalia* (Fig. 11, C, 1): side-piece rounded quadrate, broader than long; setose only on distal margin. Claspers twice as long as side-piece, with an inward, basal, spinose projection and a slender terminal tooth. Adminiculum strongly chitinized, sub-quadrate, wider than long, apically emarginate, denticulate; basal prongs stout, divaricate, truncated. Adminiculum arms with long close teeth mixed with fimbriae; moderately broad basally.

Pupa.—The species was reared from a collection of pupae with six resporatory filaments, thought to be *venustum*. Both species emerged from these pupae.. It will probably be necessary to rear *perissum* from isolated pupae to establish the presence of specific differences in the latter, if such are present.

Notes.—Two females and six males emerged with numerous adults of *venustum* on May 14–16, 1935, from pupae collected on submerged stones, tufts of grass and twigs in the Jock river, Ont. (Fig. 12, B), at a point about ten miles from Ottawa, where it enters the Rideau river. The Jock river is shallow and has a rocky bed thickly strewn with rocks and boulders. During the summer it often largely dries up in places, but in early May it was flowing swiftly over the algae-covered rocks. The temperature of the water was 62° F. I failed to find specimens among the adults of *venustum* reared from eight other rivers and streams in the Ottawa district.

Distribution.—Jock river, near Ottawa, Ont. Dyar and Shannon (5, p. 44) recorded the species from Maryland, South Carolina and Virginia.

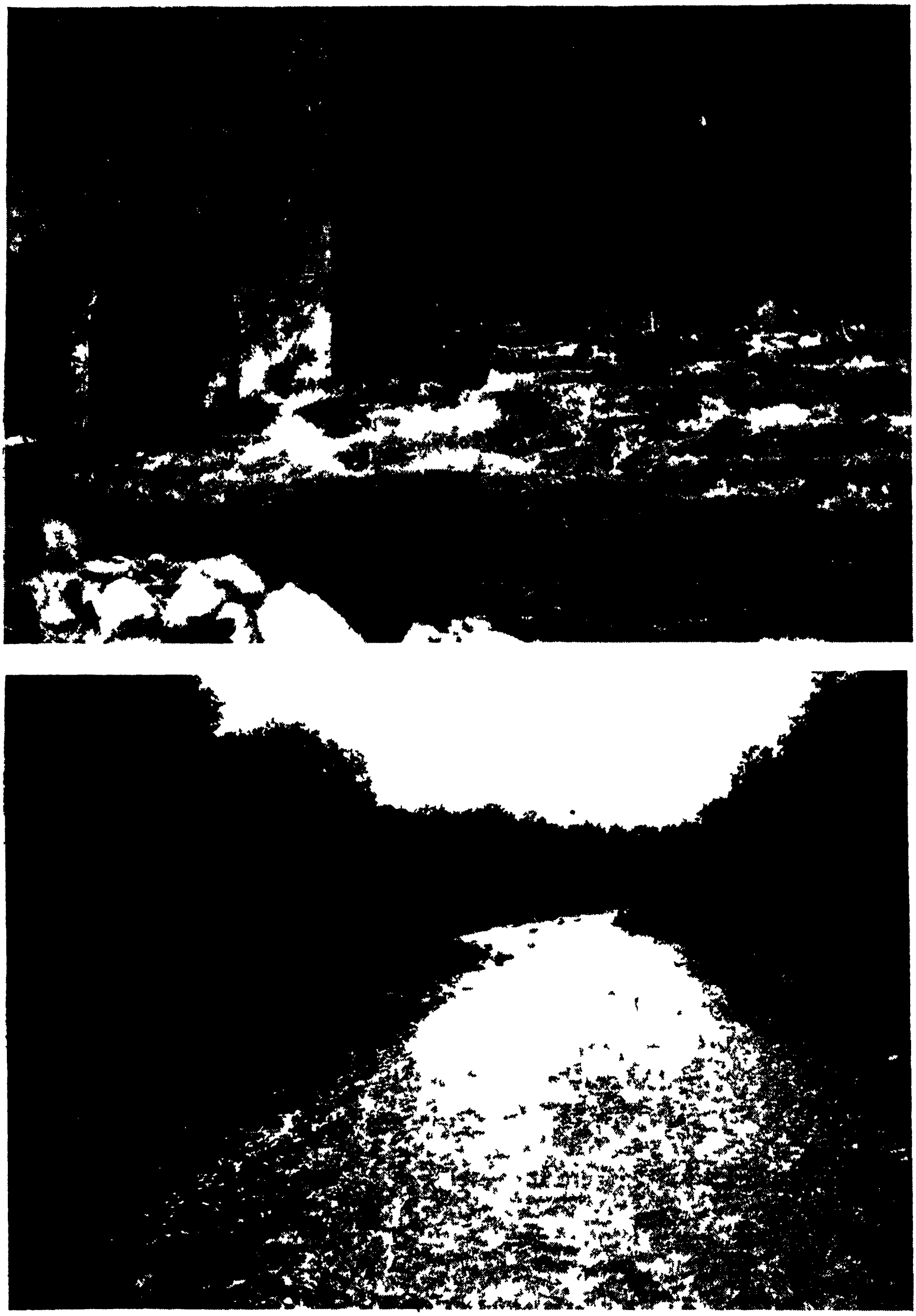

FIG. 12 *A Rapids of Blanche river below falls, south of Perkins Mills, Que Males of S. pictipes Hgn., we caught swarming here B. Jock river, near Ottawa, Ont S venustum Say, S perissum D & S and S vittatum Zett develop here*

Simulium (Simulium) fibrinflatum N. SP.

Female.—Length about 2 mm.; wing, 2.5 mm.; closely allied to *venustum* Say, from which it differs as follows: palpi distinctly longer than antennae (possibly variable); pollinosity much less thickly dusted on front and mesonotum, the integument showing through glossy black, that on the pleurae more silvery; pleural tuft black; hairs on stem vein and base of costa black; sub-costa bare; the pollinose patches on the dorsal surfaces of all tibiae more conspicuous, silvery; terminal abdominal segments glossy black. *Genitalia* (Fig. 13, A, 3, 5): ovipositor valves short, wider than long, weakly chitinized, setose, blunt tips remote. Upper part of anal lobe short, expanding medially to twice the length of cercus; narrowing, bluntly rounded and slightly produced ventrally; rather sparsely setose along posterior margin and on ventral portion. Cercus rounded quadrate; setose. Genital rod similar to *venustum*, but the tooth on chitinized expanded portion of arms longer, more slender, bluntly pointed.

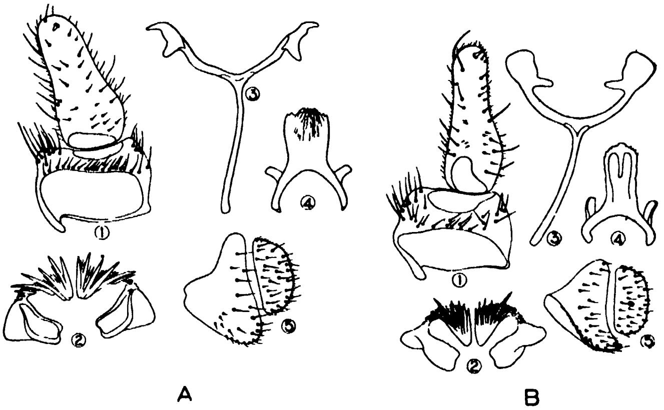

FIG. 13. *A. parts of genitalia of Simulium (Simulium) fibrinflatum n. sp.: male (1) side-piece and clasper, (4) adminiculum, (2) adminiculum arms; female (3) genital rod, (5) anal lobe and cercus. B. Parts of genitalia of Simulium (Simulium) nigroparvum n. sp.: male (1) side-piece and clasper, (4) adminiculum, (2) adminiculum arms; female (3) genital rod, (5) anal lobe and cercus.*

Male.—Similar to *venustum*, but with the following differences: pollinose band on margins of mesothorax and extending backward on disk more brilliant, iridescent anteriorly, and pearlaceous posteriorly; radial sector of wing bare of hairs except sparsely apically where it joins costa; legs paler, scarcely darker than in female: fore coxae, trochanters, femora except at apices, and the basal portions of first and second joints of mid and hind tarsi yellow, elsewhere black; the pollinose patches on dorsal surfaces of fore tibiae and bases of mid and hind tibiae conspicuous, silvery; joints of hind legs similar to female, none inflated. *Genitalia* (Fig. 13, A, 1, 2, 4): side-piece rounded quadrate, wider than long; clasper twice as long as side-piece, stout, somewhat swollen near base, a spine at tip. Adminiculum tooth-like, one-fourth longer than broad, strongly chitinized, especially the margins and basal prongs; hirsute medially; upper margin laterally denticulate; basal prongs stout, moderately divaricate, each with a short, posterior curved, sub-basal arm. Adminiculum arms with an open row of long pointed teeth against a strigate membrane; more or less pocket-shaped laterally, strigate, the inner margin more thickly chitinized.

Holotype.—Male, Ottawa, Ont., 17. VIII. 35 (reared). Can. Nat. Coll.

Allotype.—Female, same data.

Paratypes.—Fourteen females, nineteen males, 23–25. VII. and 17–19. VIII. 35 (reared).

Pupa.—Length about 3 mm.; the respiratory tuft about one-fourth as long as the pupa; the filaments in the tuft six in number, short, stout, inflated, with the appearance of a cluster of minute, semi-transparent, sausage-shaped balloons, almost sessile, branching off in the vertical plane from a short base. Cocoon about 3.5 mm., somewhat slipper-shaped, rather closely woven, with a narrow, elongate-oval, window-like aperture in each side near the anterior border. The pupa lies completely within the cocoon, the respiratory tufts opposite the "windows".

Notes.—The characteristic pupae were found for the first time on July 22, 1935, in the Remic rapids (Fig. 14, A), of the Ottawa river, at Cunningham island. The exact location (not shown in illustration) was a narrow channel dividing the east end of the island from a tiny islet through which the shallow water was rushing at a speed of about five feet per second, over a shale-rock bottom. The temperature of the water was 76° F. The infestation was largely confined to the submerged branches and leaves of sweet gale, a shrub growing in profusion along the margins of the channel, and was mixed with two other species, *nigroparvum* and *venustum*, the former predominant. Large numbers of the three species had emerged, as was revealed by the empty pupal skins. Observations had been made at this point at intervals during the spring and summer, and on the most recent visit (July 3) no pupae of *fibrinflatum* were seen, although it is probable the species was present in the larval stage. On August 16, *fibrinflatum* predominated to a striking extent, only occasional pupae of *nigroparvum* and *venustum* being found. The speed of the water had dropped to about 3.5 feet per second, and the temperature was unchanged. On August 29, the infestation consisted chiefly of larvae of the three species; a rare pupa was found on Sept. 13 (water temperature 64° F.), and none at all on Sept. 30 (water temperature 56° F.), the date of the last visit, when only very young larvae were found. Numbers of adults of both sexes were reared from the pupae collected on July 22 and August 16.

Simulium (Simulium) nigroparvum N. SP.

Simulium venustum var. *jenningsi* Twinn (not Malloch), Can. Ent. Vol. LXV, p. 3, 1933.

Female.—Average length 1.6 mm.; wing, 2.3 mm. A very small, glossy black species, similar to *venustum* in all other respects except as follows: general pollinosity less opaque; front glossy black; clypeus grayish pollinose, sub-opaque; mesonotum glossy black with faint grayish pollinosity, strongest at the margins; pubescence yellowish-brown, rather sparse, scattered, hair-like; scutellum black, marginal hairs black, recumbent pale scales absent; pleural tuft black; hairs on stem vein variable, yellow, bronzy-brown or black, or the pale and dark hairs intermixed; sub-costa bare*; terminal abdominal segments darker and more glossy. *Genitalia* (Fig. 13, B, 3, 5): ovipositor valves small, remote, weakly chitinized; setose, especially on the inner margins, which are also somewhat embrowned. Anal lobe triangular, the postero-ventral angle slightly produced posteriorly behind cercus; chitinized, moderately setose, the anterior part of basal margin clear. Cercus rounded quadrate, wider than long; setose, arms of genital rod chitinized, roundly expanded, and with a long slender tooth.

Male.—Similar in size to female, but much darker. It differs from the male of *venustum* as follows: mesonotum opaque velvet-black, the marginal pollinose band which terminates on the disk, silvery or pearlaceous, somewhat iridescent anteriorly; pilosity dark-brown, almost black; radial sector bare, except close to the apical margin at the junction with the costa; legs predominantly black, the pollinose patches on tibiae silvery; hind basitarsus about 6 × 1, and three-fifths the width of tibia. *Genitalia* (Fig. 13, B, 1, 2, 4): similar to *fibrinflatum.* Side-piece rounded quadrate, wider than long; clasper twice as long as side-piece, a slender spine at tip. Adminiculum tooth-like, twice as long as broad, densely chitinized, centrally paler, strigate; upper margin denticulate; basal prongs stout, divaricate, truncate, each with a posterior sub-basal arm. Adminiculum arms with several long slender teeth mixed with fimbriae; lateral plates broad, darkly strigate.

Holotype.—Female, Ottawa, Ont., 10. VI. 35 (reared). Can. Nat. Coll.

Allotype.—Male, same data.

Paratypes.—Seven females, nine males, Ottawa (Remic rapids), 10–18. VI. 35; one female, five males, near Perkins Mills, Que. (Blanche river), 13. VI. 35, and one male, 26. VII. 35; two females, three males, Ottawa (Rideau river), 6–7. VI. 35. All reared from pupae.

Pupa.—Average length 2.5 mm. Respiratory tuft about one-fourth the length of the pupa, consisting of ten filaments. These are arranged as follows: two main trunks arise in approximately the same plane (vertical) from a short base; the lower trunk bears two branches, each of which gives rise to three filaments, a single filament basally and a pair distally; the upper branch divides twice near the base to form two pairs of filaments, thus making ten in all. The cocoon measures about 3 mm., and is similar to that of *fibrinflatum*, slipper-shaped, with a "window" anteriorly on each side.

Synonymy.—Johannsen (23, p. 381) described a species of blackfly under the name *S. venustum* var. a, which was reared from larvae and pupae with ten respiratory filaments taken from Fall creek, Ithaca, N.Y. The adults were 1.5 mm. long, with the terminal abdominal segments shining black.

**A specimen netted near Hull, Que., September 30, 1936, had four hairs in a row on the sub-costa.*

Johannsen's figure of the pupa shows the arrangement of the respiratory filaments different from that of my specimens. The submentum of the larva of *nigroparvum*, however, is similar to that of Johannsen's var. a, and has the ventral setae three in one marginal row and four in the other. Malloch (29, p. 42) in describing *jenningsi* mentioned that among the material were adults reared from pupae at Friersons Mill, La., which were in all probability the species described by Johannsen. This seems doubtful, as Jobbins-Pomeroy (38, p. 24) described and figured the pupa of *jenningsi* as having eight respiratory filaments: these pupae were determined from adults reared from them and compared with the type. Moreöver, Dyar and Shannon (5, pp. 34, 43, 45), after examining Malloch's material in the United States National Museum, showed *jenningsi* to be a composite species made up of *venustum* Say, *slossonae* D. & S., and *perissum* D. & S. My material is specifically distinct from all of these. The species erroneously recorded by me (55, p. 3) as *venustum* var. *jenningsi* Malloch, from the Remic rapids of the Ottawa river, is *nigroparvum*.

Notes.—Pupae and larvae of *nigroparvum* were first found in the Remic rapids (Fig. 14, A), of the Ottawa river, close to the river bank on the Quebec side, near Hull, on June 25, 1931, attached to a round, smooth pole caught among the rocks in the rapids. They were next found with larvae in the same rapids on Sept. 16, 1932, on leaves and twigs of sweet gale growing on the eastern shore of Cunningham island, and hanging in the fast-running water. Numerous adults of both sexes emerged within a day or two from specimens placed in a sealer.

During 1935, visits were made to the Remic rapids twice or thrice a month from late April to the end of September. Recently transformed pupae were found on slabs of shale in the rapids on June 6 (water temperature 63° F.), and adults emerged from these June 8–10. By June 14, large numbers of empty pupal skins of this species were observed in the river; many pupae were collected and several score that emerged were preserved in alcohol. The pupae again began to appear early in July, and by July 22 (water temperature 76° F.) empty pupal skins predominated and may have marked the end of the second generation. A few pupae were found with those of *venustum* and *fibrinflatum* during visits on August 2 and 16. On August 29 (water temperature 70° F.), *S. nigroparvum* predominated in numbers over the other two species, but the pupae were not very abundant: on Sept. 13, only one pupa was found after a 40-minute search; on Sept. 30 (water temperature 56° F.) very young larvae, the species of which was not determined, were quite numerous, but no mature larvae or pupae could be found. It would seem from these observations that there are at most three generations of *nigroparvum* annually in the Ottawa region, the winter probably being spent in the immature larval stage.

Adult flies were also secured from pupae collected in the Hog's Back rapids of the Rideau river near Ottawa, Ont., June 5, 1935, and from submerged stones and grasses in the rapids of the Blanche river (Fig. 7, A), south of Perkins Mills, Que., on June 12, July 26 and August 18, 1935.

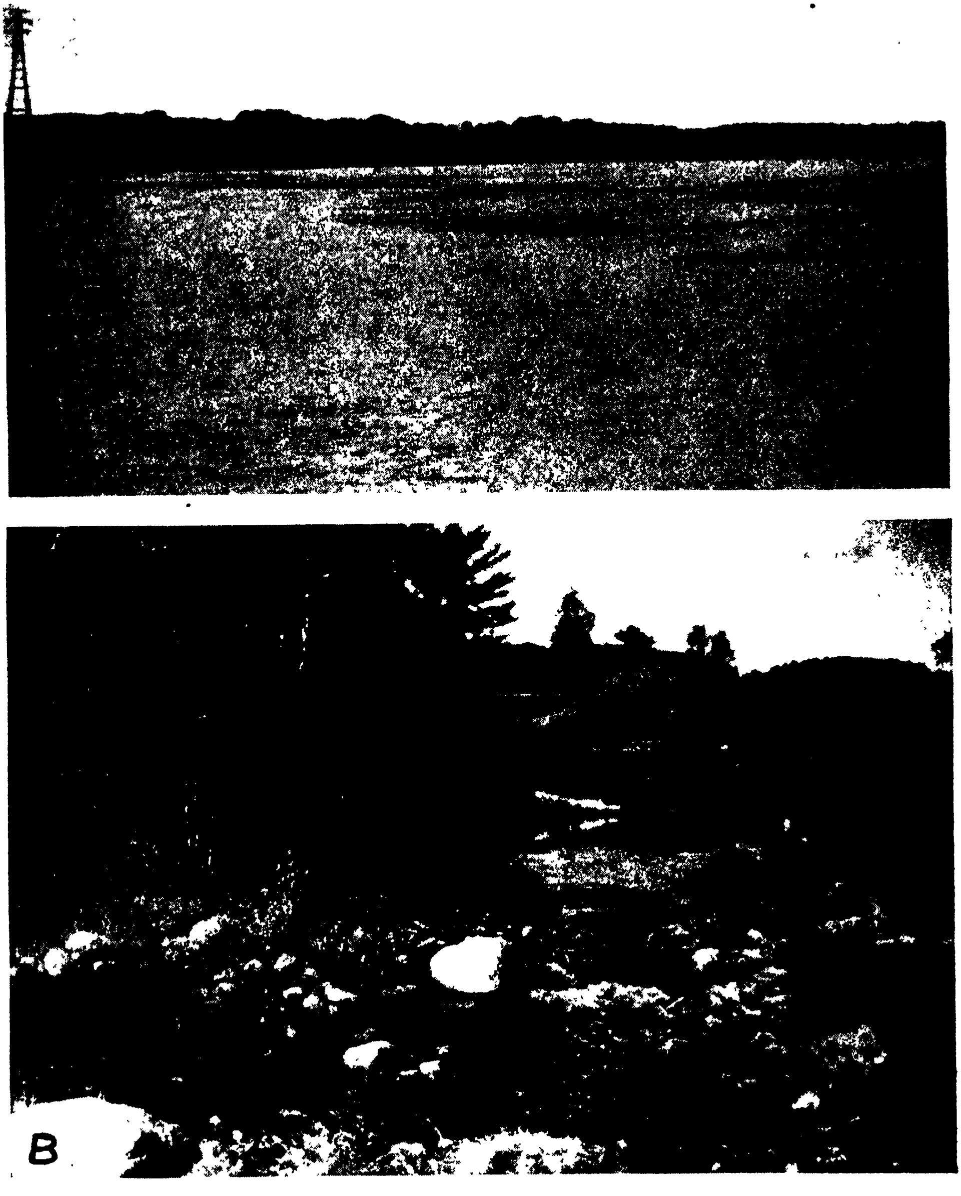

FIG. 14. *A. Remic rapids, Ottawa river; breeding place of S. venustum Say, S. nigroparvum n. sp., S. fibrinflatum n. sp. and S. vittatum Zett. B. Blanche river, north of Perkins Mills, Que.; S. venustum Say, S. vittatum Zett., S. corbis n. sp. and S. nigroparvum n. sp. found here.*

Simulium (Simulium) ottawaense N. SP.

Female.—Length, 2–3 mm.; wing, 2.8–3.5 mm. Very close to *venustum* and rather difficult to distinguish from it: somewhat larger and more robust than my specimens of *venustum;* front opaque grayish pollinose; the pale hairs on stem vein intermixed with a few dark hairs (one specimen with these hairs largely dark); terminal abdominal segments grayish-black, sub-opaque. *Genitalia* (Fig. 15, A, 4, 5): ovipositor valves conical, about as long as wide at base; weakly chitinized on inner margins; evenly setose. Anal lobe dorsally about one-third length of cercus, expanding to twice its length ventrally, broadly and roundly produced; bare on anterior margin, a few spicules below; elsewhere more or less evenly setose. Cercus rounded quadrate, a little wider than long; setose. Arms of genital rod moderately divaricate, angulate, outward expansions each with lower margin extended into a broad, long, roundly-pointed tooth.

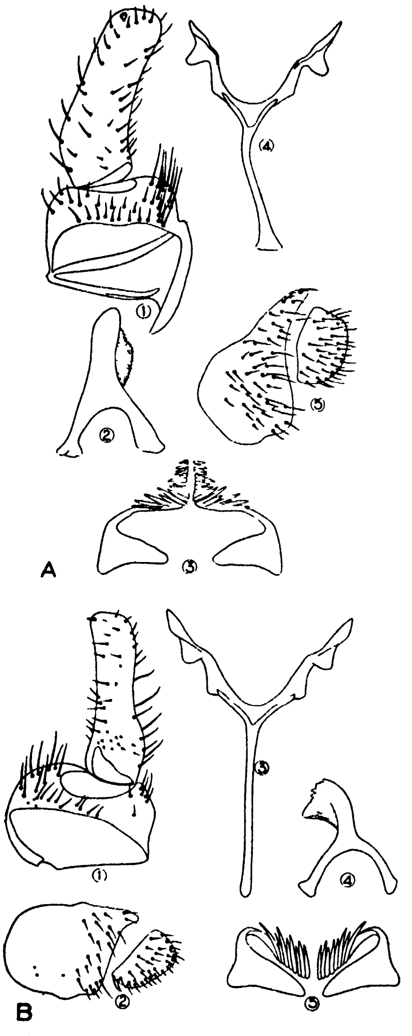

FIG. 15. *A. Parts of genitalia of Simulium (Simulium) ottawaense n. sp.: male (1) side-piece and clasper, (2) adminiculum, (3) adminiculum arms; female (4) genital rod, (5) anal lobe and cercus. B. Parts of genitalia of Simulium (Simulium) corbis n. sp.: male (1) side-piece and clasper, (4) adminiculum, (5) adminiculum arms; female (2) anal lobe and cercus, (3) genital rod.*

Male.—Very similar to the male of *venustum*, but general appearance somewhat paler, the white pollinosity on the thorax more opaque, the legs less heavily chitinized (observable in cleared specimens.) *Genitalia* (Fig. 15, A, 1–3): side-piece rounded quadrate, nearly as long as wide, reinforced with a transverse bar of denser chitin as figured. Clasper broad, somewhat strap-shaped, a little more than one and one-half times as long as side-piece; a spine at tip. Adminiculum inverted Y-shaped, strongly chitinized; narrow, laterally compressed, with a rounded, setulose, posterior process or keel; basal arms stout, oblique, truncated. Adminiculum arms held on a convex membrane; chitinized, slender medially, and with a row of rather numerous, close-set short to long teeth; broad laterally.

Pupa.—Length about 4 mm. The respiratory tuft consists of eight filaments, and is slightly more than one-half the length of the pupa. The respiratory filaments arise from a short main trunk: six are in pairs, one upper pair and two lower; two are unpaired. One of the unpaired filaments arises directly from the apex of the main trunk, the other from near the base of the upper stalk. The stalks bearing the two lower pairs of filaments are unequal in length, the upper being about one-half that of the lower. The cocoon is of the wall-pocket type; loosely woven, especially anteriorly, the anterior border not thickened to form a rim.

Holotype.—Male, Pinks lake, near Hull, Que., 26. VII. 35 (reared from pupa). Can. Nat. Coll. (slide).

Allotype.—Female, Carleton Place, Ont., 7. VIII. 35; other data as above.

Paratypes.—Seven females, five males (reared from pupae).

Notes.—The type material was reared from pupae collected in the Leamy creek close to its source, at Pinks lake, in the Gatineau hills, near Hull, Que., and in the Mississippi river, at Carleton Place, Ontario. In the Mississippi river (Fig. 9, B) several pupae, and numerous larvae, were found (Aug. 5) with *vittatum* on pieces of *Myriophyllum* entrapped in the fast-flowing water below a concrete dam in the town. The temperature of the water was 73° F. Adults emerged, August 6–9. The Leamy creek, at Pinks lake, is a tiny stream flowing down a wooded hillside. The gradient is slight close to the lake where the collection was made, and the flow was only about one foot per second over the stone and debris-strewn bottom. The water temperature was 76° F. (July 24). Scattered pupae and pupal skins of *vitattum* and an occasional pupa of *aureum* were also present. Adults emerged on July 25–26. I have collected pupae of this species in late May and June from several streams in the Ottawa district, during recent years, but had not previously reared the adults. I also found them in the Madawaska river, Algonquin Park, Ontario, on June 30, 1931. The larvae and pupae usually occur in mixed infestations with *venustum* and *vittatum*.

Simulium (Simulium) corbis N. SP.

Female.—Length about 2.2 mm.; wing, 3.4 mm. Close to *venustum*, and very similar in size, appearance and external characters, but differing as follows: antennae almost entirely black, the basal joints only faintly reddish; pubescence on front, vertex and mesonotum yellow, somewhat more scale-like; upright marginal hairs on scutellum pale yellow and black; hind basitarsus about 5 × 1; claws with a small but distinct, pointed, sub-basal tooth. *Genitalia* (Fig. 15, B, 2, 3): ovipositor valves about as long as wide, tips rounded; largely membranous, slightly embrowned on inner margins; minutely setulose; each with a median group of spicules. Anal lobe quadrate, rounded anteriorly; as long as ninth tergite, and one and one-half times as wide as cercus (vertical width); minutely setulose; posterior third moderately setose; a group of tiny spicules antero-ventrally; dorsal and ventral margins each with a small, minutely spiculate, rounded protuberance. Cercus rounded quadrate, wider than long; setose. Arms of genital rod angled on lower margins; outward expanded portion more strongly chitinized, and with a broad, blunt tooth.

Male.—Similar in all respects to the male of *venustum* except as follows: pleural membrane reddish, tarsal joints of all legs entirely black; hind basitarsus about 4 × 1 and four-fifths width of tibia. *Genitalia* (Fig. 15, B, 1, 4, 5): side-piece and clasper similar to *ottawaense*; side-piece rounded quadrate, about as long as broad, a reinforcing chitinous bar present, but less distinct; claspers about one and one-half times as long as side-piece, broad, constricted slightly at middle, the setae on undersurface unusually long and stout; a slender spine at tip. Adminiculum narrow, strongly chitinized, inverted Y-shaped viewed dorsally; produced posteriorly into a flat, apically rounded process or keel, sparsely and minutely setulose on the sides, and marginally denticulate, the denticles backwardly directed, sharply pointed, sub-equal; basal arms stout, oblique, truncated. Adminiculum arms on a supporting convex membrane, chitinized, and with a row of rather long teeth; the arms broadening and triangulate laterally.

Holotype.—Female, Blanche river, near Perkins Mills, Que. (reared). Can. Nat. Coll.

Allotype.—Male, same data.

Paratypes.—Ten females, four males, same data.

Pupa.—Length about 3.5 mm.; respiratory tuft approximately three-eighths as long as the pupa. Each tuft consists of ten filaments of about equal length and thickness, arising in sessile pairs from a short, common base, and held fanwise, all more or less in the vertical plane. The cocoon is slipper-shaped, about 5 mm. long; the posterior two-thirds rather closely woven: anterior to this portion, on each side, is an elongate opening next to the anterior border; extending beyond the border, except narrowly dorsally, and forming the cuff of the slipper is an interlacing network of filaments, like the ornamental rim of a basket. The pupa lies in the cocoon with the respiratory tufts protected by this basket-work extension, through the meshes of which the head and respiratory filaments are visible.

Notes.—The pupae were found on May 22, 1935, on the submerged stems of dogwood growing close to the bank of the Blanche river, in the rapids, a short distance below a waterfall, five miles south of Perkins Mills, Que. (Fig. 7, A). The river was flowing swiftly over a stony bottom; the temperature of the water was 53° F. Pupae of *venustum* and *quebecense* occurred on stones nearby. Eighteen males and twelve females of *corbis* had emerged by May 26; several were damaged and preserved in alcohol-glycerine, the others pinned.

Acknowledgments

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TETRAMERES CRAMI SWALES, 1933, A NEMATODE PARASITE OF DUCKS IN CANADA.

MORPHOLOGICAL AND BIOLOGICAL STUDIES[1]

By W. E. Swales[2]

Abstract

The male of *Tetrameres crami* is described and the female is redescribed in the light of further studies. The life history has been elucidated and the larval stages described. Two fresh-water amphipods, *Gammarus fasciatus* Say and *Hyalella knickerbockeri* (Bate) are intermediate hosts. A list of definitive hosts, notes on the ecology of the intermediate hosts and on tetrameriasis crami, are included.

The original description of *Tetrameres crami* was based on the morphology of nine female specimens collected from a domestic duck from Ottawa, Ontario. Since that time this nematode has been found to be the most common and probably the most important helminth parasite of domestic ducks in the province of Quebec. It has also been found as a parasite of 12 species of wild ducks from both eastern and mid-western Canada. Morphological studies of a large amount of material have shown that the original description of the female must be considerably modified, and this redescription, together with a description of the male, has been included herewith.

The studies on the life history, which involves an amphipod as intermediate host, indicate that this nematode is probably widespread in North America.

Morphology

Family Tetrameridae Travassos, 1914

Tetrameres. Female body globular or spindle-shaped, with four longitudinal furrows corresponding to the median and lateral lines. Mouth of male generally with two lateral trilobed pseudolabia and a dorsal and a ventral interlabium; female lips indistinct or lacking. Buccal capsule with chitinoid walls. A slender muscular oesophagus surrounded by the nerve ring and followed by a more robust glandular oesophagus.

Tetrameres crami Swales, 1933

Male.—Filiform, 2.9-4.1 mm. long by 70–92μ wide. Cuticle adorned with two double rows of spine-like sensory organs which extend from the anterior end to the cloacal region. The "lips" conform to the description of those of *T. americana* and *T. fissispina* by Chitwood and Wehr (1), there being two lateral trilobed pseudolabia and two inconspicuous interlabia. Two papillae are situated at the base of each interlabium, one on each side; one smaller papilla is situated in the middle of each pseudolabium, slightly above the others. The cleft or spear-shaped cervical spines extend from the anterior part of the head as modified lateral alae; they are 60μ in length. Buccal capsule roughly bell-shaped and strongly chitinoid, 15–20 μ. long by 9–11μ wide. Muscular oesophagus (11) 0.281–0.325 mm. in length, surrounded near its middle by a prominent nerve ring. Glandular oesophagus 0.730–0.875 mm. long by 42–56μ in maximum width. The cervical papillae, distinct from the spine-like sensory organs, may be equidistant from the anterior end of the body or one slightly anterior to the other; the ranges of distances are 0.116 mm. and 0.122 mm. to 0.145 mm. The tail, of more or less constant length in most nematodes, is rather variable; distances from the cloacal aperture to the tip range between 0.148 mm. and 0.248 mm., the commonest distance being 0.195 mm. Specimens from wild ducks frequently showed a greater tail length than those from domestic ducks but this was later shown to be due to the fact that the former birds were seldom obtained in a fresh state and that slight decomposition caused minor deformities in the nematodes. The tip of the tail is in the form of a cuticular cone, 10μ in length.

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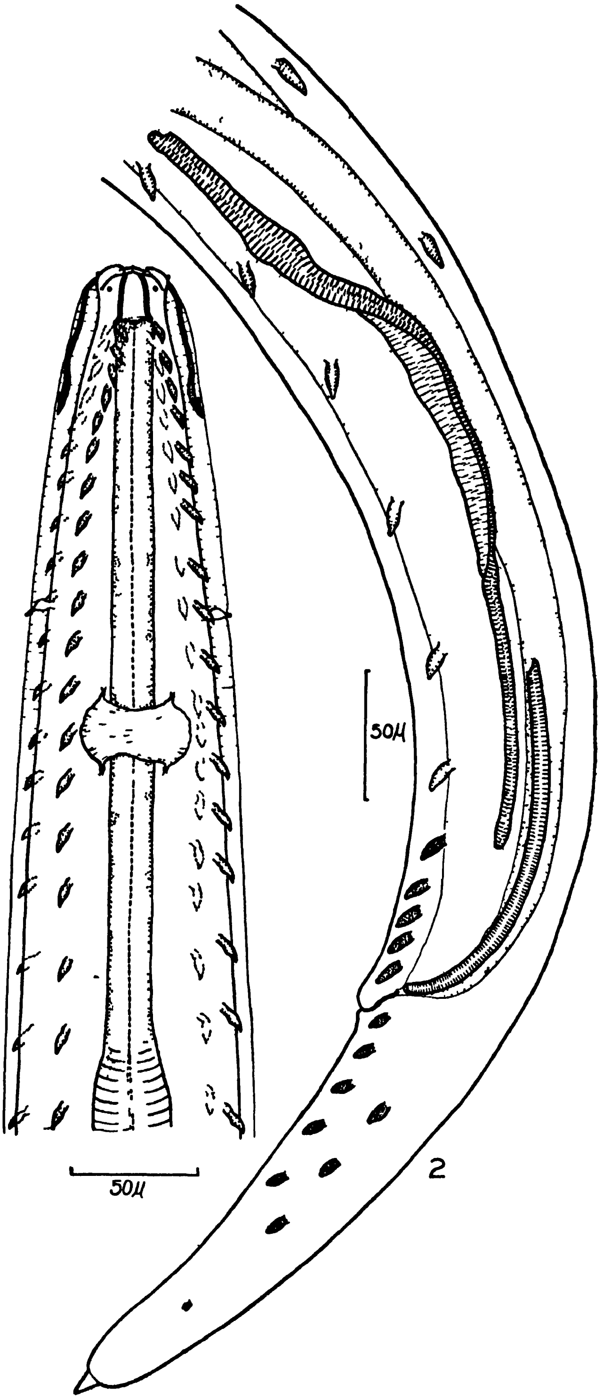
Spicules unequal, not strongly chitinoid; the right one narrow and curved, 0.136–0.185 mm. in length. The left spicule larger and twisted, 0.272–0.350 mm. in length. Testis about 2.0 mm. long, reflexed at a point about 0.150 mm. posterior to the oesophagus; vas deferens robust, about 0.8 mm. long, the junction with the testis being conspicuously constricted.

The cuticular processes, the "spines" of other described species, appear to be all sensory organs, there being a process from each "spine" which passes through the cuticle into the hypodermis; all the tips are refractive, and increasingly so towards the posterior end of the body. With the advice of Dr. B. G. Chitwood, who kindly examined some of my specimens, I have tentatively differentiated the posterior 14 pairs as genital papillae, on account of their position, the larger refractive tips and decreased length. These papillae are distributed as five pairs preanal and sub-ventral, one pair adanal, four pairs postanal and sub-ventral, and four pairs in a lateral position on the tail. The posterior pair of the "lateral" four pairs are small and inconspicuous. The adanal papillae are postanal in some specimens, thus giving these individuals five pairs of postanal papillae in the sub-ventral rows. The somatic sensory organs are somewhat longer, have smaller refractive tips, and number from 49–55 in each row; they range from 10μ long at the anterior end of the body to 18μ long in the region just anterior to the spicules. Postanal genital papillae 8–9μ in length, with the exception of the last pair which are much smaller.

Female.—Globular to spindle-shaped, 1.5–3.25 mm. in length by 1.2–2.2 mm. in maximum width. Live specimens blood-red in color and able to change their shape very considerably by waves of contraction and expansion. The anterior end protrudes from the globular body for a distance of 0.84 mm. in fully grown specimens, and the protruding posterior part is 0.5 mm. long. Cervical papillae approximately 0.214 and 0.241 mm. from the anterior extremity.



FIGS. 1 AND 2. 1 *T crami, adult male, head end.* 2. *Adult male, caudal end. The genital papillae shaded more heavily than the somatic sensory organs.*

Buccal capsule or head capsule sub-circular to barrel-shaped, 12–14μ long by 11–12.2μ wide. Muscular oesophagus 0.22–0.247 mm. long, surrounded just posterior to its middle by the nerve ring; glandular oesophagus 0.916–1.3 mm. long. Intestine saccular, about 2.0 mm. long by 1.0 mm. wide in fully grown specimens. Anus 0.113–0.156 mm. from the tip of the tail. The tail ends in a small cuticular cone.

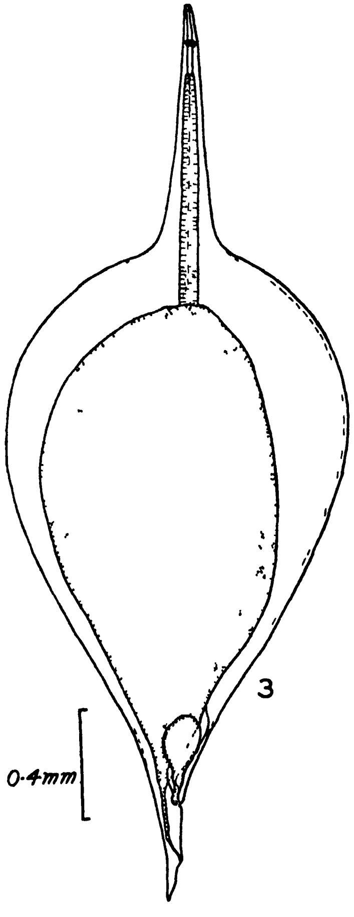


FIG. 3. *T. crami, adult female.*

Vulva situated in a deep depression in the posterior projection near the body proper, 0.319–0.350 mm. from the caudal extremity. The short vestibule of the ovejector is joined on its ventral side by a copulatory receptaculum at a point 50μ from the vulva. Receptaculum thick-walled and saccular, 0.3 mm. long by 0.15 mm. wide. The trompe bifurcates at a point ventral to the intestine and 0.5–0.6 from the vulva, and the branches join the uteri, which together with the long filiform ovaries, fill the body cavity. Eggs oval and fully embryonated when laid; they are 41–57μ long by 26–34μ wide, the commonest size of 200 specimens measured being 53μ long by 28μ wide.

Primary Hosts:

Anas boschas domesticus,
Anas platyrhynchos,
Anas rubripes,
Chaulelasmus streperus
Mareca americana,
Nettion carolinense,
Querquedula discors,
Spatula clypeata,
Dafila acuta,
Nyroca marila,
Nyroca affinis, and
Glaucionetta clangula.

Intermediate Hosts:

Gammarus fasciatus Say and *Hyalella knickerbockeri* Bate.

Location:

Proventriculus (glandular stomach).

Distribution:

Canada (Provinces of Quebec, Ontario, Manitoba, Saskatchewan and Alberta) and probably throughout North America.

The Life Cycle of *T. crami*

The search for the intermediate hosts of this nematode was confined to aquatic arthropoda owing to the fact that the adult parasite was only found in ducks of the Subfamilies Anatinae and Fuligulinae. Because of the prevalence of *Daphnia pulex* and because this crustacean had been reported as an intermediate host of *Tetrameres fissispina*, large numbers of this species were exposed to feces of infested birds and to heavy suspensions of embryonated ova. *D. pulex* ingests the ova readily but in no case have the ova been found to hatch, but are passed out unchanged. Examination of approximately 200 specimens which had been suitably exposed some days previously failed to reveal the presence of larvae. Other Entomostraca tested and found negative are *Daphnia longispina*, *D. magna*, *Daphnia* sp., *Ceriodaphnia* sp. and several species of Cladocera.

Following the tests of the Entomostraca a search for Amphipoda revealed the presence of two common species in this district, *Hyalella knickerbockeri* (Bate) and *Gammarus fasciatus* Say. Two specimens of the former species were exposed to a suspension of ova obtained by dissection of female nematodes. Seven days later these amphipods were found to have several active larvae in their body cavities.

A number of specimens of *Gammarus fasciatus* was similarly exposed and subsequent examination revealed that all the specimens contained larvae, some being very heavily infested. After five or six days the larvae can be readily seen in most specimens of both of the above-mentioned amphipods by examining the live animal under a dissecting microscope. Data concerning the development of the parasite in the intermediate hosts were obtained from a series of experimental infestations of both intermediaries.

The ova, which are infective when laid, are readily ingested by the amphipods in water. Ova stored in water at 4–5° C. for ten months were found to be still viable, and were used for experimental infestations. They hatch in the digestive tract and migrate to the body cavity. In 12 to 18 hours the larvae are easily found by dissection of the amphipods; measurements of these first stage larvae have shown them to be 0.197 mm. to 0.223 mm. in length by 12μ to 13μ in width. At this stage the larvae are very inactive and rapidly die and disintegrate in water. They are comparatively thick, their anterior ends being rounded and transversely striated; details of morphology are difficult to determine, but the head end is blunt, with no lips, and the tail end is bluntly rounded.

In from seven to eleven days after exposure second stage larvae are seen in the body cavity. These forms are from 0.279 mm. to 0.95 mm. in length by 39μ in maximum width and can be distinguished by the rounded caudal extremity; under the cuticle of this extremity can be seen the caudal papillae of the third stage (Fig. 4). The size to which this stage can grow before it moults appears to be very variable, as third stage larvae slightly more than 0.45 mm. in length and 33μ in width have been found. The second stage larvae, after the period of activity and growth, migrate to various parts of the body and become encysted. Common locations of the cysts are the coxal plates and on the inner surface of the dorsal shield; in some cases they have been observed among the muscles of the peraeopods, apparently interfering with normal muscular activity. The cysts are in the form of thin-walled and egg-shaped formations loosely attached to the inner surfaces of the chitinoid structures of the amphipod. They are approximately 0.33 mm. by 0.27 mm. in size; they are easily ruptured by slight pressure of a cover-slip. The encysted larvae assume various coiled or "figure-eight" positions, which they frequently change.

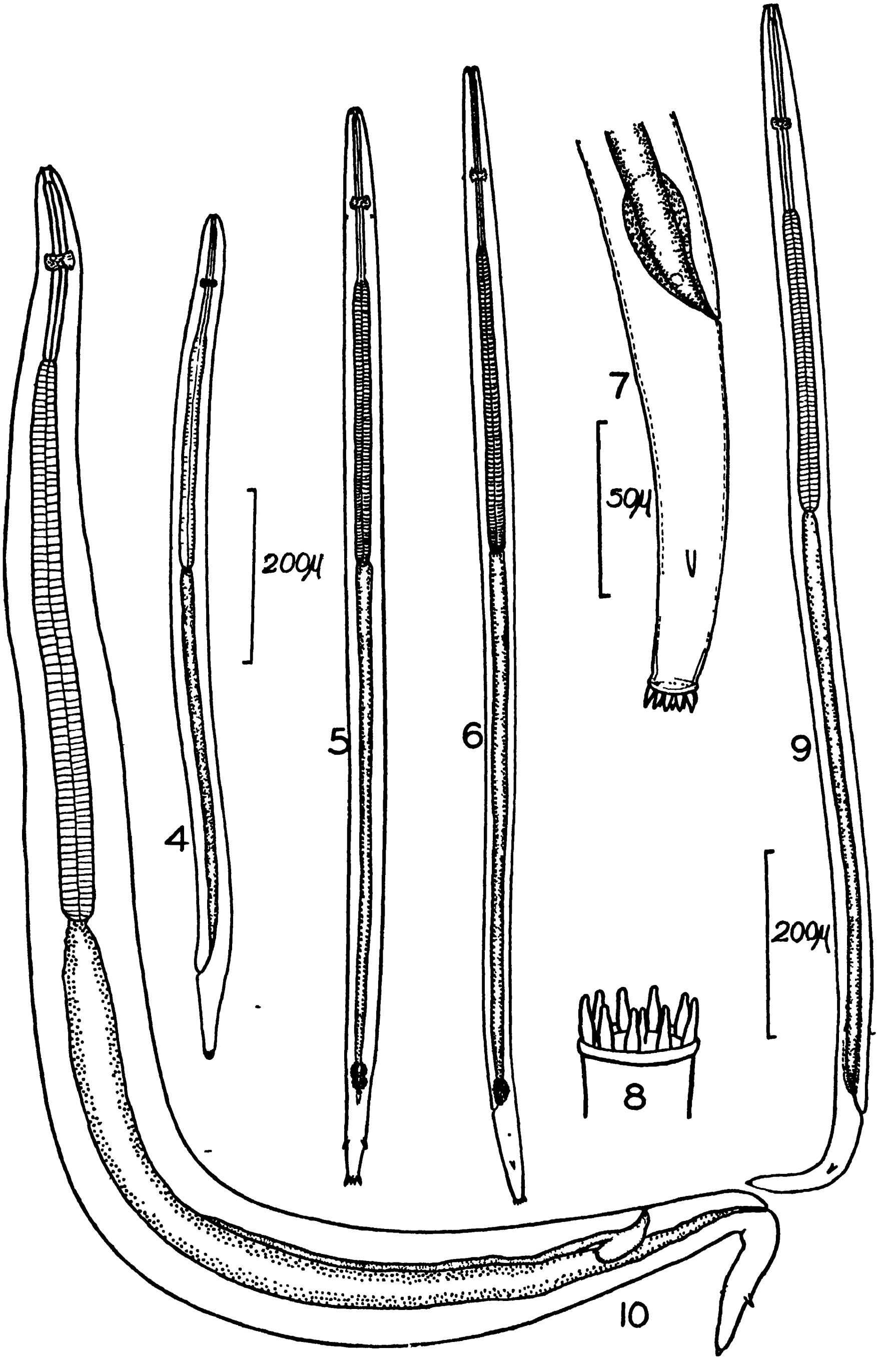
Although it is the rule for the second stage larvae to encyst, instances have occurred in experimentally infested amphipods of a moult to the third stage prior to encystment. Usually, however, the moult occurs within the cyst, and development continues in this position.

The third stage encysted larvae have been found to vary greatly in size, 0.455–1.406 mm. in length by 33–45μ in width. These measurements were recorded from specimens encysted in *Hyalella knickerbockeri* for from 20 to 65 days, but the largest larva (1.406 mm. long) developed in a second generation *H. knickerbockeri* in 24 days. This rapid growth of the encysted larva has not been found to involve another moult and each specimen had the general structural characteristics which appear to be constant within the stage. The anterior extremity has four oral papillae; the cervical papillae are approximately 0.130 mm. from the anterior extremity. Buccal capsule cylindrical, 11–11.8μ long by 5–6μ wide. Muscular oesophagus 0.152–0.211 mm. long; glandular oesophagus 0.268–0.390 mm. long by 25μ in maximum width. Tail 0.097–0.120 mm. long, gradually tapering to a wide tip. Situated laterally on the tail 50μ from the tip is a pair of spine-like sensory organs about 14μ long, similar in outline to those on the body of the adult male, and having a fine basal process and a refractive tip. The end of the tail is cylindrical, and is about 13μ in diameter; it is adorned with a circle of nine papillae situated on the inner edge of a cuticular ring, and one papilla is situated on the tail end in the centre of the ring (Fig. 7). The outside papillae are of equal length (6–8μ), the central one being somewhat smaller.

The genital primordium is readily seen in this stage as a small mass near the end of the intestine.

DEVELOPMENT IN THE FINAL HOST

Experiments involving infestation of domestic ducks and "tame" mallards (*Anas platyrhynchos*) were conducted to demonstrate the infectivity of the third larval stage and to complete cross infection experiments from wild to domestic ducks and *vice versa*. The life cycle was experimentally completed from *Anas rubripes* through *G. fasciatus* to *Anas platyrhynchos*, from *A. rubripes* through *G. fasciatus* to *Anas boschas domesticus*, from *A. boschas domesticus* through *G. fasciatus* to *A. boschas domesticus* and from *A. boschas domes-*



FIGS. 4–10. 4. *Second stage larva, from body cavity of Hyalella knickerbockeri, ten days after ingestion of ova.* 5. *Third stage larva, from Gammarus fasciatus, 32 days after infestation. (Same scale as Fig. 4.)* 6. *Third stage larva from the proventriculus of a naturally infested domestic duck. (Same scale as Figs. 4 and 5.)* 7. *The caudal end of a third stage larva from H. knickerbockeri, 60 days after infestation.* 8. *A diagrammatic illustration of the papillae on the end of the tail of a third stage larva.* 9. *Fourth stage larval male from the proventriculus of a naturally infested duck.* 10. *Fourth stage larval female. (Same scale as Fig. 9.)*

ticus through *H. knickerbockeri* to *Anas platyryhnchos*. The domestic ducks and the mallards used in the experiments had been hatched in an incubator and raised in this laboratory. Details of the experiments are as follows:—

Experiment I

Ova obtained by dissecting female tetrameres from the proventriculus of *Anas rubripes*, were placed in a pint jar of water containing a number of *G. fasciatus*. Five days later (November 4, 1935), the amphipods were removed to a jar of clean water, examination of four having revealed the presence of larvae in their body cavities. On November 18, 1935, six specimens were examined and found to contain encysted larvae; these amphipods were placed in a gelatine capsule and fed to a domestic duck. No nematodes were found on autopsy on December 14, 1935.

Experiment II

Five *G. fasciatus*, from the same lot as those in Experiment I. which were seen to contain encysted larvae, were fed to a domestic duck on November 24, 1935. Autopsy on December 27, 1935, did not reveal any nematodes in the proventriculus.

Experiment III

Ova from nematodes in *A. rubripes* were fed to *G. fasciatus* on November 7, 1935. The amphipods were removed to clean water 24 hours later. On December 7, 1935, two of these amphipods, one apparently containing one encysted larva and the other ten, were fed to a seven-month-old mallard. This bird was killed and autopsied on January 7, and eight female tetrameres ranging in size from 1.03 mm. by 0.578 mm. to 2.48 mm. by 1.07 mm. were found in the crypts of Lieberkühn. In the mucous lining were found one immature male, 1.5 mm. long by 47μ wide, and one-third stage larva. The females did not contain embryonated ova but in other respects were essentially the same as those obtained from natural infestations.

Experiment IV

Two *G. fasciatus* from the same lot as those in Experiment III were seen to contain several encysted larvae. These amphipods were fed to a domestic duck on December 12, 1935 (33 days after ingestion of ova). The duck was killed and examined on February 11, 1936, and one adult female tetramere and an immature male were recovered from the proventriculus.

Experiment V

Numerous specimens of *H. knickerbockeri* were exposed to ova freshly dissected from females obtained from *Anas rubripes* on November 5, 1935. Ten of these amphipods, each apparently parasitized by one encysted larva, were fed to a domestic duck on December 15, 1936. The autopsy on January 11, 1936 did not reveal any helminths.

Experiment VI

On November 8, 1935, several *G. fasciatus* were exposed to ova obtained from female tetrameres from a naturally infested domestic duck. Three of these amphipods, each apparently containing one encysted larva, were fed to a domestic duck on December 15, 1935. The autopsy on January 11, 1936, revealed one sexually immature female tetramere, 1.7 mm. long by 1.0 mm. wide.

Experiment VII

On November 8, 1935, numerous specimens of *H. knickerbockeri* were exposed to ova from tetrameres from a naturally infested domestic duck. Five of these amphipods were fed to a mallard on January 13, 1936. This bird was killed on February 15, 1936 and one mature male tetramere, three mature females and one immature female tetramere were recovered. The male was 3.4 mm. long by 89μ wide, tail 0.193 mm. long, spicules 0.330 mm. and 0.162 mm. long. The mature females were 1.5 mm. by 1.0 mm., 2.2 mm. by 1.5 mm. and 2.0 mm. by 2.0 mm. in size.

Experiment VIII

Ten infested specimens of *H. knickerbockeri* from the same lot as those in Experiment VII were fed to a two-month-old chicken in June, 1936. The autopsy ten days later failed to reveal any tetrameres.

In the above experiments all the ducks used were free of helminths other than *T. crami*. Owing to the fact that they had never had access to water other than the filtered laboratory supply, controls were dispensed with. The lack of uniformity of development and the failure of the duck in Experiment V to become parasitized appears to indicate individual resistance.

The experiments indicate that the third stage larvae require some time for growth in their encysted state before they are infective for ducks. They can be infective after 29 days in the intermediate hosts and it is probable that infectivity may be attained in a somewhat shorter period. The sexually mature adult state can be reached in the proventriculus of ducks in 33 days after ingestion of infective larvae. However, owing to the previously noted irregularity in growth-rate in the experimentally infested ducks, it seems inadvisable at this time to state definite periods necessary for development.

The fourth stage larva does not conform to the description of that of *T. fissispina* by Seurat (8). I have determined this stage to be the form found in the crypts of Lieberkühn in which the character of the tail-ending has been changed from the ring of papillae to a cone-shaped organ similar to that of the adult; it does, however, retain the lateral sensory spines. This stage exhibits marked sexual differentiation, the male is similar in outline to the adult male, and the female body is wide and the enlarged intestine usually contains some ingesta. The fourth stage larval male is 1.36–1.41 mm. in length by 36–48μ wide; the buccal capsule roughly cylindrical, 13–14μ long; muscular oesophagus 0.17–0.2 mm. long, glandular oesophagus 0.330–0.386

mm. in length. Tail 0.13–0.15 mm. long, ending in a cuticular cone 8–9μ in length. Sensory spines of the tail 0.08–0.1 mm. from the caudal tip. The outlines of the genital structures were not clear in the available specimens.

The fourth stage larval female is dark in color owing to the intestinal mass. The available specimens measured 1.7–1.93 mm. long by 0.120–0.132 mm. wide. Buccal capsule cylindrical, 14μ long and somewhat wider than that of the male. Muscular oesophagus 0.2–0.23 mm. long; glandular oesophagus 0.53–0.6 mm. long. Tail 0.12–0.13 mm. long, ending in a cuticular cone 8–9μ long. The sensory spines approximately 70μ from the caudal tip. A single outstretched genital tube, up to 0.46 mm. long, joins a small undeveloped copulatory receptaculum near the rudimentary vulva which is covered by the larval cuticle.

In considering the forms described above as the fourth larval stage, several points worthy of note were taken into consideration. The form described as the third stage and infective larva grows considerably in size and has been found to live for a considerable time in both the intermediate and definitive hosts. Although many specimens have been examined no evidence of a moult to another similar stage has been observed. The general structure of all these specimens has been the same, but each organ has shown evidence of growth. By analogy, it would appear that the form described by Seurat (8) was the third larval stage of *T. fissispina*. The stage described above as the fourth, however, does exhibit characteristics that indicate a necessary moult to the adult state although, except in the region of the vulva of the female, the larval cuticle is not well marked. The sensory spines are probably the best criteria of this stage, as it is unlikely that these organs could be cast off without a complete ecdysis. Unfortunately I have been unable to find a specimen in the act of its final moult.

Only adult males and third stage larvae have been found in the mucus of the proventriculus; thus it is apparent that the fourth larval stage exists entirely in the crypts and that only the adult male returns to the surface.

The immature adult female quickly assumes the globular shape and the specimens observed are slightly tinged with red and considerably shorter than the fourth stage larvae. The longitudinal furrows are well marked. Sensory organs on the tail are lacking.

T. crami COMPARED WITH OTHER KNOWN SPECIES OF THE GENUS

A consideration of the description of the adult male, the modified morphology of the female and the studies on the life history reveal that *Tetrameres crami* is more closely related to *T. fissispina* than to any of the other known species. The descriptions of the latter species by various authors are somewhat contradictory. According to Wharton (*vide* Cram (2) and Hsü (7)), *T. fissispina* is a much larger species; according to Travassos (14), this species is of similar size but differs considerably from *T. crami* in the length of the female tail, the sizes of the buccal capsules, the length of the male oesophagus and the spicule lengths, as well as in other minor points. *T. crami* has not been found as a parasite of *Gallus gallus*, which is a common host of *T. fissispina*. All attempts to infest *Daphnia pulex*, a reported intermediate host of *T. fissispina*, with *T. crami* have failed.

The other known North American species of *Tetrameres*, *T. americana* Cram, 1927 and *T. pattersoni* Cram, 1933, cannot be confused with *T. crami*. Both are very much larger species, and have gallinaceous birds as definitive hosts and Orthoptera (grasshoppers and cockroaches) as intermediaries.

T. paucispina Sandground, 1928, from a South American "black-bird", differs chiefly in the length of the male tail. All other species have distinct structural differences as well as different hosts. *T. fissispina*, *T. americana*, *T. pattersoni* and *T. crami* are the only species of the genus for which the life cycles are known.

Tetrameriasis Crami

The effect of *T. crami* upon the definitive host has not yet been fully determined, but a study of the histopathology of tetrameriasis crami is being continued.

In infestations involving the presence of one hundred or more female nematodes in the crypts of Lieberkühn the host has always been found in rather poor condition. In the few cases studied from this aspect, sections of the crypts have revealed the adult parasites to be enclosed in a thin fibrous cyst around which little or no evidence of acute inflammatory processes can be seen. However, the crypt is greatly distended and, owing to mechanical blockage, the secretory glands cannot function. As the function of the proventriculus is to bathe the passing food with gastric juice and pepsin, and thus break down protein, severe infestations probably impair this process to some extent. This effect alone may account for the failure of the parasitized birds to gain weight normally.

The females of this genus are known to feed on blood, this fact having been substantiated in the case of *T. crami* by examinations of intestinal detritus. The intake of blood for food requirements must be fairly extensive in the case of an adult egg-producing female. The toxic action decribed in tetrameriasis fissispina of pigeons by Timon-David (12) may also be found to play a part in this disease. Cram (3) notes that in tetrameriasis americana of *Gallus gallus* there is a catarrhal condition associated with a thickening of the walls of the proventriculus. This condition has been noted in a few cases in this study, but it is not constant in heavy infestations with *T. crami*.

The alternate orientation of the females in the crypts in heavy infestations of *T. fissispina* of pigeons as described by Timon-David (12), has not been observed in the case of *T. crami*.

Notes on the Intermediate Hosts

Gammarus fasciatus and *Hyalella knickerbockeri* have a wide range of distribution in North America, and are rather abundant in many bodies of water.

Gammarus fasciatus is found commonly on sandy shores of rivers and lakes. In this district of the Province of Quebec it is easily collected in the late summer and autumn by sweeping a sieve through aquatic vegetation in from 12 to 18 inches of water. The most favorable habitat observed in 1935 was a favorite feeding ground of wild ducks; this habitat is a shallow sandy bay on Lake St. Louis, containing an abundance of aquatic vegetation chiefly represented by *Vallisneria americana*, *Elodea canadensis*, *Potamogeton Richardsonii*, *Potamogeton filiformis* and *Chara* spp. A few days prior to the freezing over of the shallow water, masses of these plants were washed up near the shore, and large numbers of *G. fasciatus* were collected from this vegetation. Even after most of the water along the shore was frozen over, small open holes yielded hundreds of these amphipods. These animals were kept alive for some weeks in the laboratory in glass tanks provided with sand, clear water and *Vallisneria americana*.

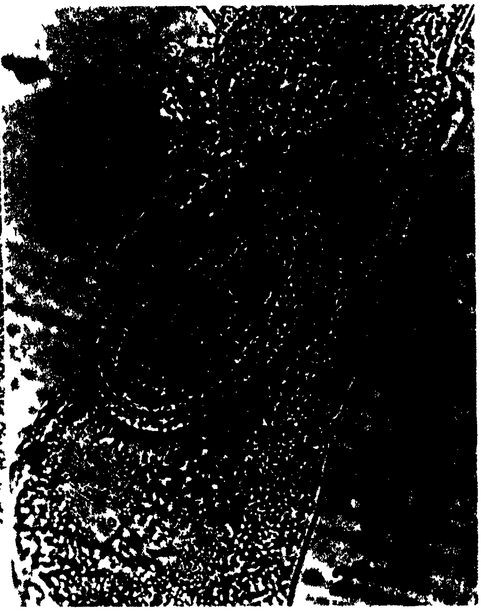
G. fasciatus becomes infested with *T. crami* very readily but the second stage larvae appear to exert a marked pathogenic effect upon this amphipod. In heavy infestations the animal becomes yellow in color and so feeble that it may be imprisoned by the surface film. Many of my specimens which contained third stage infective larvae exhibited the same symptoms of weakness and this would make them easy prey for the final host under natural conditions.

Hyalella knickerbockeri is usually found in an entirely different habitat from that mentioned above. It is a common inhabitant of ditches, creeks, quiet muddy bays of lakes, small rivers and ponds. Quantities of algae appear to be essential for its optimum condition of life, although dead leaves of trees may also serve as food. A handful of filamentous algae from a muddy shore of a creek will usually yield many specimens of this amphipod. It is very easily kept alive in the laboratory, and second generation specimens are readily obtained if the adults are placed in a small jar with mud and algae. Changes of water are undesirable and aeration is unnecessary. Specimens have been kept alive in a pint jar for over ten months and are still living, water having been added monthly to replace that lost by evaporation.

H. knickerbockeri is easily infested experimentally with *T. crami*. They do not become so heavily infested as *G. fasciatus* however, even under identical conditions of exposure; the largest number of larvae found in a single specimen was four. This amphipod does not appear to be affected to any visible extent by the infestation; infested specimens have been kept in aquaria for over five months and on being dissected at the end of this time yielded active larvae.

Of the above-mentioned intermediate hosts, *G. fasciatus* appears to be the one chiefly responsible for the infestations in wild ducks, owing to the fact that it is the species commonly present on the typical feeding grounds of scaups and other diving ducks. On the other hand, *H. knickerbockeri* is probably responsible for the infestation of domestic ducks because these birds do not usually have access to sandy shores of large lakes but they frequently feed in ponds, creeks, etc.

PLATE I



3

4

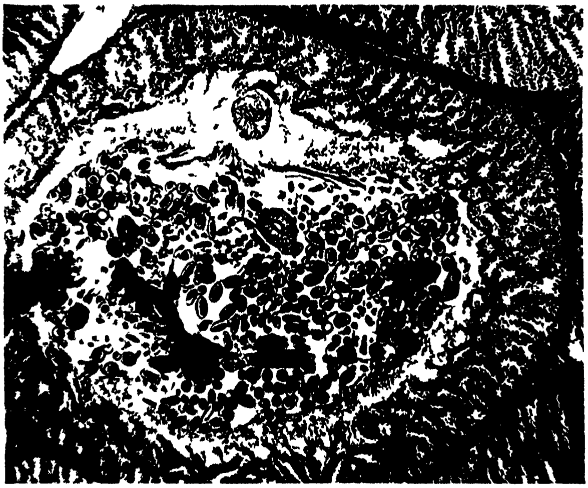


Plate I *A third stage larva encysted in a peraeopod of Gammarus fasciatus* (×110) 2 *A third stage larva encysted on a thoracic somite of Hyalella knickerbockeri* (×143) 3 *Gammarus fasciatus* Say (×5) 4 *Hyalella knickerbockeri (Bate)* (×3 5) 5 *Section of a female of T crami in a crypt of the proventriculus of a duck*

Both *G. fasciatus* and *H. knickerbockeri* form an important part of the food of many fishes. A single adult stickleback *(Eucalia inconstans)* placed in a tank containing *H. knickerbockeri* was found to have ingested ten of these amphipods in one hour. The fact that so many of our waters have been badly depleted of fish may explain the prevalence of the amphipods and hence the prevalence of *Tetrameres crami*. Readjustment of the aquatic fauna in areas where tetrameriasis crami is found to be of economic importance should tend to prevent harmful infestations of ducks.

Attempts to destroy amphipods by chemical means have not shown any promise. For example, a 1% solution of copper sulphate killed all the amphipods in one hour, but a 1 : 500,000 solution failed to kill a mixed lot in 24 hours; these tests were conducted in jars free from organic matter.

Acknowledgments

I wish to express my appreciation of the co-operation of Dr. Maurice C. Hall, who found and sent to me some obscure literature on *T. fissispina*. It is my pleasure to acknowledge as well the help of Dr. B. G. Chitwood, who advised me regarding some important structures in the adult nematodes, of Dr. E. Melville DuPorte, Macdonald College, who identified the specimens of *Gammarus fasciatus*, of Dr. M. W. Smith, Atlantic Biological Station, St. Andrews, New Brunswick, and Miss Thelma Wood, Brown University, Rhode Island, who sent me living cultures of *Daphnia pulex* and *D. longispina* respectively.

Messrs. Henry Gatehouse and Son, Inc., Montreal, rendered invaluable assistance in supplying quantities of viscera of ducks; without this material the work would have been severely handicapped.

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STUDIES ON THE ENDOPARASITIC FAUNA OF TRINIDAD MAMMALS

IV. FURTHER PARASITES FROM TRINIDAD DEER[1]

BY THOMAS W. M. CAMERON[2]

Abstract

The trematode, *Paramphistomum cotylophorum* is recorded from *Mazama simplicicornis*, and the female of the nematode *Eucyathostomum longesubulatum* is described.

Since the publication of my last paper on the parasites of the Trinidad deer, *(Mazama simplicicornis)* Professor F. W. Urich has collected five other specimens of this host. These were fixed in formalin in the usual manner but only the intestines and liver were sent to the Institute for examination. Seven species of helminths—one trematode, one cestode and five nematodes—were recovered; all except one being found in the intestine.

Paramphistomum cotylophorum FISCHOEDER, 1901
(SYN: *Cotylophoron cotylophorum*)

This trematode was found in some numbers in two of the deer, in both cases in the duodenum. Most of the specimens were minute, but some were fully developed and contained a small number of eggs; none however had reached its maximum size. Even in the very small specimens in which the genitalia was still a rudiment, the genital sucker was visible and conspicuous. The larger specimens were sufficiently mature to show the excretory canal crossing the Laurer's canal and to show the two lobed testes, situated tandem to each other.

The only amphistome which appears to have been previously recorded from this host is *P. liorchis* described in 1901 by Fischoeder from Brazil. In this species, not only is the genital sucker absent, but the testes have a smooth contour.

The finding of *Paramphistomum cotylophorum* in these deer is of considerable interest. It would seem that it is an African parasite (also found in India) which has been imported to America with cattle. It has recently been recorded from St. Lucia, B.W.I. by Metivier (4) and from the southern

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United States, although there is no previous record from South America. It is probable that the Trinidad deer are picking up their infection from domestic ruminants introduced from Africa or Asia.

Moniezia benedeni (Moniez, 1879)

This cosmopolitan cestode was tentatively recorded from this host by the writer last year (1). It is now definitely recorded on the basis of a well preserved specimen from one of these animals.

Setaria bidentata (Molin, 1858) R. & H. 1911

This filarid worm was recorded from this host early this year (2). Another specimen was recovered from one of the present animals.

Eucyathostomum longesubulatum Molin, 1861

Two males of this interesting nematode were recorded from a deer in an earlier paper. Five males and nine females were recovered from two animals in the present series. The males differed in no way from those previously described (2).

The females had an average length of 16.5 mm., the largest being 17.0 mm. Like the males, they were of a deep brown color. The head end differed in no way from the male. The tail however (Fig. 1) is long and tapering, the anus being situated about 1.1 mm. from the gently rounded tip. No caudal papillae were observed.

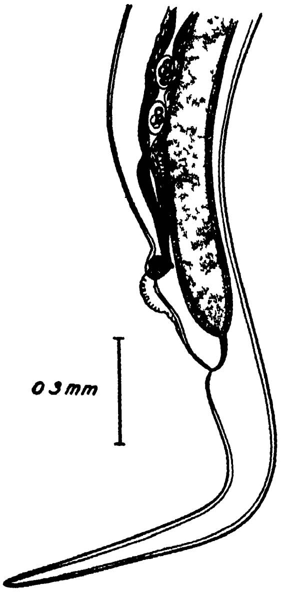

Fig. 1. *Eucyathostomum longesubulatum. Tail region of female.*

The anus is generally situated in a deep depression; in a single specimen, this depression was absent. The rectum is a simple tube, but its junction with the intestine is very abrupt and the posterior end of the intestine, in all cases, was very voluminous and sac-like, with thick walls.

The vulva is situated about 0.35 mm. anterior to the anus. Posterior to the vulva is a conspicuous cuticular swelling, concave on its anterior face and presenting a pectinated appearance when viewed in optical section. This swelling gradually becomes smaller laterally and disappears at about the level of the lateral lines. The actual vulvar opening lies at the middle of the concavity on the anterior aspect of this swelling. The anterior lip of the vulva is also swollen, this swelling being smaller, however, and fitting into the concavity; it is more granular and less hyaline than the posterior swelling and seems to be formed mainly from the sub-cuticular layer. Its anterior margin is abrupt, and from this point the body rapidly expands to its maximum thickness. The vulva and both swellings are generally covered with a coating of cement as in the case of the Oesophagostomes and Sclerostomes.

The vagina is long and simple. Its total length is about 2 mm., of which the terminal third forms the *pars ejectrix*. It is directed anteriorly, and communicates with two voluminous parallel uteri which run forward to about the middle of the body where they join the ovarian tubules. These occupy the same portion of the body as is occupied by the uteri, only a few loops being seen anterior or posterior to them.

Nematodirus urichi CAMERON, 1935

This trichostrongyle was described in 1935 (1) on the basis of three females and a single male. A considerable number of examples was secured from the present animals. A careful examination showed that they did not differ from the types, except that the heads of the females, like those of the males, were usually inflated. This inflation terminates at the level of the cervical papillae and is not striated.

Mazamastrongylus trinitatis CAMERON, 1935

Several specimens of this trichostrongyle were found in two of the deer, but they differed in no way from the type specimens.

Strongyloides papillosus (WEDL, 1856)

A single specimen of *Strongyloides* was recovered from one animal. It was 3.25 mm. long with a maximum width of 0.07 mm. The oesophagus measured 0.8 mm. in length and the mouth was surrounded by four papillae. The vulva was situated 1.25 mm. from the tip of the somewhat fingerlike tail (which was 0.07 mm. long). It does not seem to differ in any significant respect from *Strongyloides papillosus* of ruminants and it is accordingly referred to that species.

This nematode is quite cosmopolitan in its distribution and is one of the commonest parasites of sheep in Canada. It has previously been recorded from sheep from South America.

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ON THE FOURTH STAGE LARVA OF *CHABERTIA OVINA*[1]

By Henry J. Griffiths[2]

Abstract

The structure of the early fourth stage larva of *Chabertia ovina*, a common parasite of ruminants, is described in detail.

Although the free-living stages of *Chabertia ovina*, an important nematode of ruminants, have been described by Cameron (1) in 1926, Morgan (4) in 1930, Mönnig (3) in 1931 and Dikmans and Andrews (2) in 1933, the early parasitic larval stages do not appear to have received any attention.

On post-mortem examination of a domestic goat, *Capra hirca*, a number of early fourth stage larvae was obtained from the contents of the large intestine. This goat had been pastured in a small paddock for experimental purposes, and was slaughtered in the month of December, several weeks after cold weather had commenced. A number of parasites was obtained from the stomach and intestinal contents. The abomasum yielded a large number of trichostrongyles and ciliates, the duodenum many trichostrongyles, and the large intestine a few adult *Chabertia*, several Oesophagostomes, as well as the early fourth stage larvae mentioned above.

All the larvae collected were at approximately the same stage of development. No transitional stages were observed between the young fourth stage larvae and the adults. As the goat had presumably acquired his recent infection a short time previous to his death, varying transitional stages from the very early fourth stage larvae to the mature adult would have been expected. The lack of these may possibly be attributed to some resistance reaction on the part of the host.

Technique

All larvae were fixed *en masse* in 5% formol saline. When necessary, they were examined in lacto-phenol.

In order to examine the cephalic region of the larvae *en face*, the head was cut off with a razor blade under the dissecting binocular microscope, just posterior to the junction of the buccal capsule and the oesophagus. The buccal capsules were then dehydrated in 95% alcohol, covered with a large drop of Euparal mounting medium and oriented as desired under a cover glass. Before examination under the oil immersion lens, the mounts were allowed to dry for at least 24 hours.

This technique proved invaluable for the *en face* examination of the buccal capsule, the consistency of the mounting medium permitting orientation of this small object in whatever position was desired.

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Contribution from the Institute of Parasitology, McGill University, Macdonald College, P.Q., with financial assistance from the National Research Council of Canada.

[2] *Research Assistant.*

Fourth Stage Larva

These larvae show an unusual uniformity in size, development and general characteristics. All were very early fourth stage and the only variation observed was in the slight difference of degree of development of the provisional buccal capsule.

The average length of the larva is 1.79 mm. and the average breadth, taken just posterior to the junction of the oesophagus and intestine, is 0.083 mm.; any extremes vary little from the mean.

The cuticle is thick and transversely striated, the striations becoming more pronounced in the posterior region of the body. About the middle of the oesophageal region is a cervical groove or cuticular flap. This flap is ventrally placed and when viewed laterally, appears as a very pronounced expansion of cuticle; when examined from a ventral aspect, it is a fan-tail in outline.

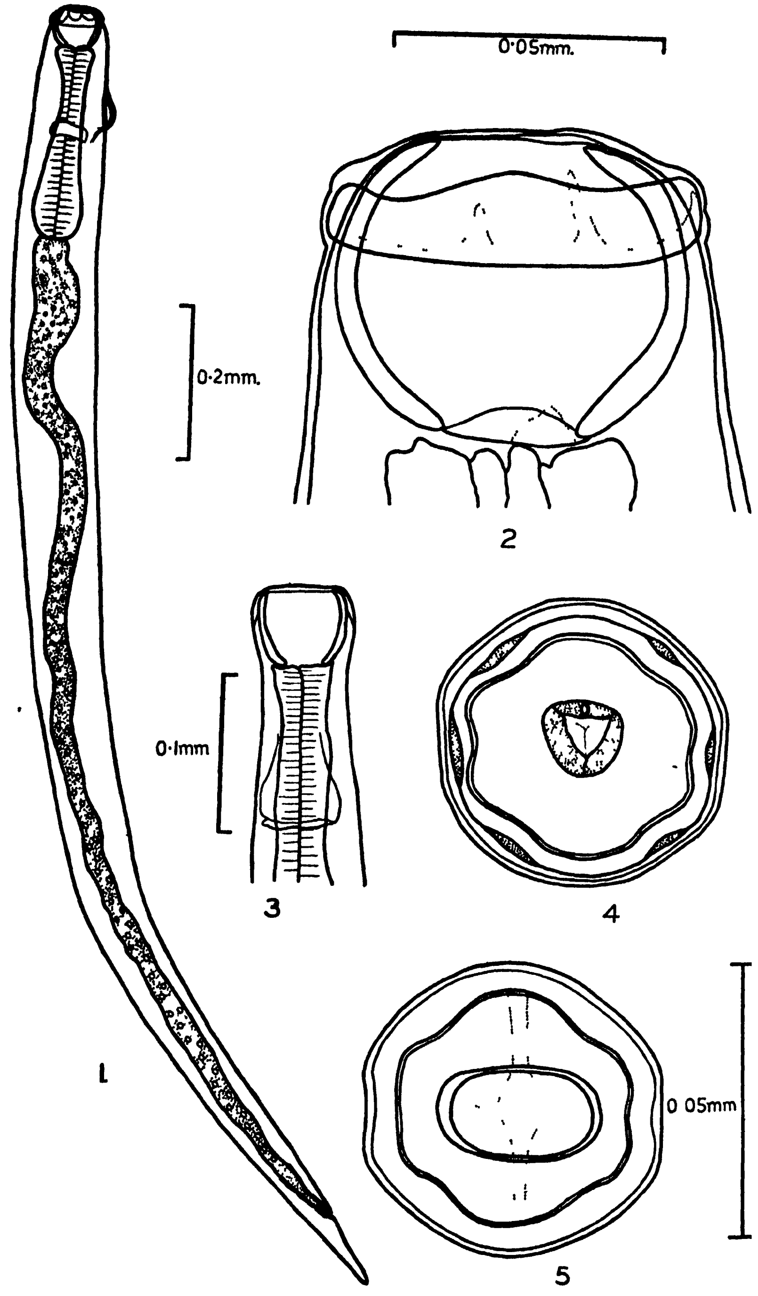
The mouth capsule is almost spherical. The aperture is directed anteriorly, oval in shape and unguarded in any manner. In the immature fourth stage, its average inside diameter is 0.048 mm. It is not completely surrounded by a capsule wall, the latter being absent at the anterior and posterior end of the cavity (Fig. 2).

At the anterior end of the capsule, the body cuticle swells to form an epaulette-like enlargement. An internal cuticular collar is also present.

In one specimen only, probably more immature than the others, the buccal capsule, when viewed from a postero-lateral aspect, showed a bell-shaped cuticular structure, the apex of the bell being directed posteriorly and leading by a fine tubule to the lumen of the oesophagus. The complicated oesophageal armature of the infective larva, as described by Cameron (1), consisting of a set of three cuticularized, triangular plates, is probably the rudiment of the provisional buccal capsule. This bell-shaped structure would appear to be the anterior region of the oesophagus of the infective larva, gradually being forced anteriorly and discarded, to be replaced by the muscular oesophagus of the young adult as the formation of the provisional buccal capsule proceeds.

Several buccal capsules were mounted vertically and examined *en face*. Examination revealed the presence of two openings. One is the true oval mouth capsule, the greater axis of the oval being laterally situated; the other, inside of the first, is a spindle-shaped aperture, with its greater axis dorsoventrally disposed (Fig. 5). The latter structure was observed at varying stages, and in a few instances was found to be rupturing laterally. The purpose of this excessively thin cuticular lining is not understood, but it has undoubtedly had some protective function preparatory to the formation of the provisional buccal capsule.

Located on the cuticle on the exterior of the provisional buccal capsule, are three pairs of papillae carrying nerve endings. One pair is lateral, the others being dorso-lateral and ventro-lateral in position.



C. ovina, 4th stage larva. FIG. 1. *Side view of entire larva.* FIG. 2. *Lateral view of buccal capsule.* FIG. 3. *Ventral aspect of anterior region.* FIG. 4. *Optical section of oesophageal-buccal capsule junction. Same scale as Fig. 5.* FIG. 5. *Optical section of mouth opening.*

The *en face* view (Fig. 4) of the base of the buccal capsule shows the outer cuticular wall, the thick hyaline wall of the capsule on the exterior of which are located the papillae bearing nerve endings. In the centre of the drawing is shown the junction of the oesophagus to the base of the capsule; the triradiate muscular walls of the lumen of the oesophagus are apparent, the dorsal wall bearing a papilla on which is the opening pore of the oesophageal gland.

A dorsal and a ventral projection, situated on the same plane as the nerve papillae, were observed in a few specimens but their presence was by no means consistent. Other very small projections were also observed, situated on the same plane as those located dorsally and ventrally, but their presence and positions were not consistent in different specimens, in some individuals being entirely absent.

The average length of the oesophagus is 0.255 mm. and the average diameter 0.057 mm. It is quite similar in shape and structure to that found in the adult. At the junction of the oesophagus and intestine may be noted the three oesophago-intestinal valves, these structures only being observed in a few specimens. The nerve ring in the majority of specimens is very indistinct and difficult to observe without excessively high magnification. It appears as a band about the middle of the oesophagus, just posterior to the cervical groove.

Cervical and cephalic glands were not observed. Immediately posterior to the cervical groove and ventral is the minute excretory pore, with its small duct which soon becomes lost in the oesophageal region.

The intestine is quite typical and terminates in the rectum, the walls of which appear to be considerably thickened. The simple anus is about 0.09 mm. from the tip of the tail.

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STUDIES ON THE BIONOMICS AND CONTROL OF THE BURSATE NEMATODES OF HORSES AND SHEEP

III. FURTHER OBSERVATIONS ON THE TOXICITY OF URINE AND SOME RELATED SUBSTANCES FOR SCLEROSTOME LARVAE IN FECES[1]

By I. W. Parnell[2]

Abstract

Comparative tests with cow urine show that there is considerable variation in its lethal effect on horse sclerostome larvae in manure. Urine gradually loses its lethal power over a period of months and is useless after a year. Tests on urines of various farm animals are also recorded, as well as on fluid from byres and manure heaps. The chemical composition of urine is discussed and tests with ammonia and acetone on larvae are described.

The first paper of this series (12) demonstrated the possibility of employing natural urine as an agent for sterilizing feces against the free-living stages of bursate nematodes. It did not however give data that would permit urine to be compared, quantitatively, with other possible agents, and accordingly a new series of experiments was commenced in the autumn of 1935.

Preliminary experiments, recorded in the first paper, had suggested that cow urine was as effective as horse urine for this purpose but the first set of experiments in the present series, made (as are all the experiments reported in this paper) with 40 grams of horse feces, showed no lethal effect. The control experiment (xxxix in Table IV) showed 35,000 larvae and in cultures to which as much as 25 cc. of cow urine was added, 32,000 larvae developed. The urine used had an unknown history, so urine from ten known cows was collected separately and tested under similar conditions. The results of these tests are shown in Fig. 1.

In order to enable these results to be readily followed, this, and many of the following figures, as well as similar ones in subsequent articles, have been prepared in the form of a block graph on a logarithmic scale. This shows small numbers, which are important, on a much larger scale than the large numbers, which are relatively unimportant. As zero cannot be shown on such a scale, and, as anything less than unity in the present work must be zero, small blocks are drawn below the bottom line to indicate those cases in which no larvae at all were recovered. In each case the numbers are those obtained from 40 grams of horse feces. The controls are *not* shown in these graphs but a roman numeral at the top of each column gives the reference to the control, the details of which are given in full in Table IV. In all cases, the numbers of larvae obtained in the control cases are significantly large—approximately equal to or greater than those obtained in the actual test; any exceptions to this are specially noted. It was not found possible to express

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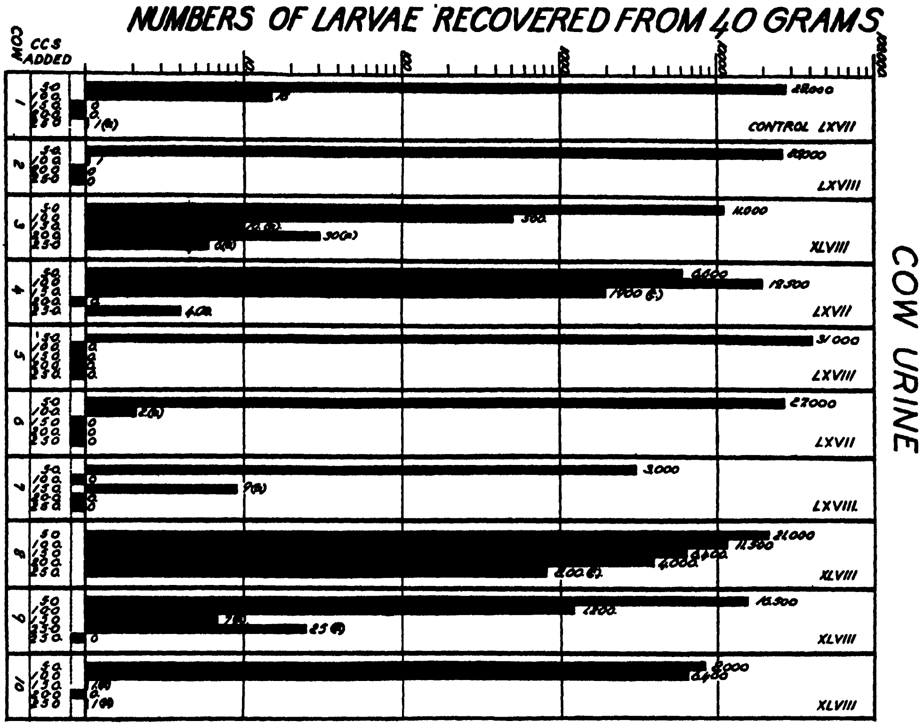

FIG. 1. *Cow urine. The Figure shows the results of tests with samples of urine from 10 cows. Roman numbers refer to the controls shown in Table IV. (a) After a number means that all larvae seen were dead, (b) that most were dead, and (c) that some were dead. The letters have the same significance in subsequent figures.*

these figures as percentages of the control figures and so make accurate comparison of various quantities of various dilutions possible. Too many unknown factors are yet involved in development and, until these are properly understood, percentages would be misleading. The graphs, however, do show the points at which the substance tested is comparatively or absolutely lethal.

In the case of five of these ten cows, 10 cc. of fresh urine was sufficient to completely sterilize the feces; three others required 15 cc. and one 20 cc. to produce the same result. In one case, even 25 cc. was insufficient to kill all the larvae, although it definitely reduced the numbers present. This particular animal was an aged Ayrshire with a very high milk record, but was nearly dry at the time of the test. She was five months pregnant and was reported to have had a diseased kidney a few years previously. No relation between breed, duration of pregnancy or age and lethal effect of the urine was noted in the other donors.

These variable results suggest why previous workers (3, 5, 6, 14) have had contradictory results. As will be seen from a subsequent paper, synthetic urea is very lethal and it may be that these irregularities may be traced to a varying urea content of natural urine.

Samples of urine from some of these cows were retained in the laboratory for varying periods and tested under similar conditions. These results varied slightly (Table I) but showed that the potency gradually declined, although urine was still sufficiently lethal to have some practical value even when five months old. The control in each case (cxi) gave 17,500 larvae.

TABLE I

Fresh and stale cow urine

Quantities required to destroy all larvae in 40 grams.

Cow	Fresh	Stale	
4	20 cc.	25 cc.	15½ weeks old†
5	10 cc.	15 cc.	15½ weeks old‡
6	10 cc.	15 cc.	15½ weeks old
7	10 cc.	10 cc.	15½ weeks old
9	15 cc.	25 cc.	24 weeks old
10	15 cc.	20 cc.	24 weeks old

† *75 larvae survived 15 cc. urine.*
‡ *4 larvae survived 10 cc. urine.*

Two other cows of known history, were selected and their urine tested fresh, eight weeks and 24 weeks old. A second sample was collected from each and tested fresh and when 15 weeks old. The results of these tests are shown in Fig. 2. In the first set of tests Urine A was consistently non-lethal while Urine B was lethal at first but gradually lost its potency. On the second test, however, Urine A when fresh had acquired lethal properties, which persisted to some extent at the end of 15 weeks; Urine B, on the other hand, had lost some of its lethal effects.

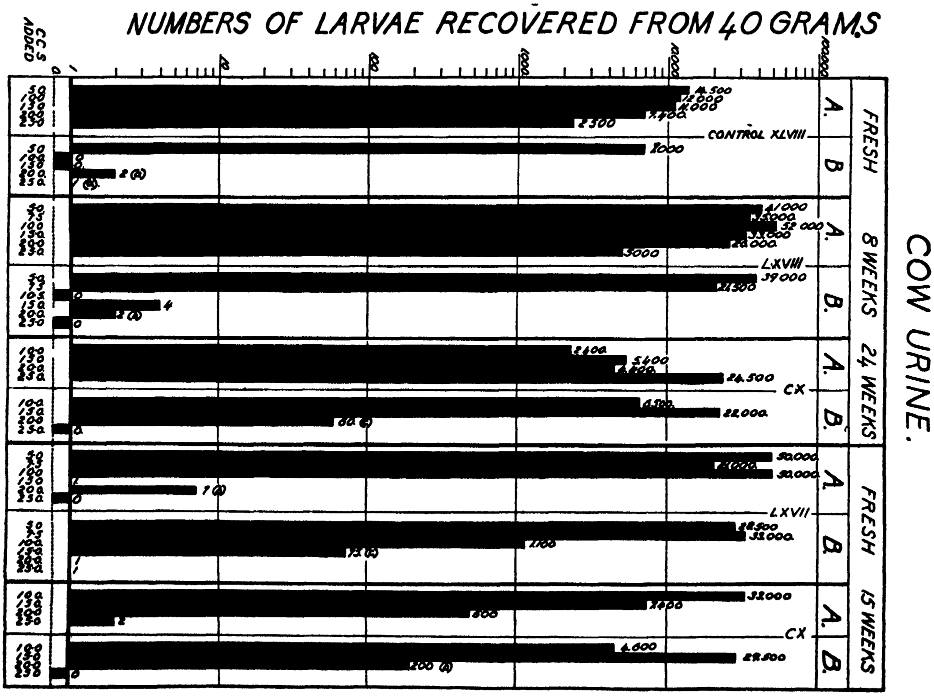

Fig. 2. *Cow urine. Comparative tests with urine of two cows collected on two occasions and tested after different intervals. Cow A was a Shorthorn on a high ration, "dry" but 8 months pregnant on the first occasion, but after calving and milking on the second occasion. Cow B was an Ayrshire, pregnant, but milking for six months and on a high ration on the first occasion, but "dry" on the second.*

Horse urine was diluted with varying quantities of water and its effect tested on standard weight cultures. These experiments showed that the amount of urine required to sterilize feces is roughly proportional to the dilution. Thus in one typical series when 7.5 cc. of fresh undiluted urine was necessary, 15 cc. of 1 : 1, 20 cc. of 1 : 2 and 25 cc. of 1 : 3 dilutions was required.

Horse urine, like cow urine, was found to lose its potency gradually. Of the samples of urine referred to in the previous paragraph, double the quantity was required to sterilize the feces when it was 28 weeks old.

Tests on both cow and horse urines over a year old showed that urine of that age was practically without any lethal properties.

Urine from a number of other species of animals was similarly tested with horse feces. Only a small number of samples of each was tested and no critical conclusions can, in consequence, be drawn.

Human urine varied in its potency, even from the same donor, but of six samples tested on the usual 40-gram samples, five required 20 cc. (although in one of these cases, 15 cc. of fresh urine permitted only 40 larvae to survive compared with a control of 9,500). In the sixth sample, 25 cc. was necessary. These results suggest possible applications of this method to human hookworm control, assuming of course, that hookworm larvae are not more resistant to urine than are sclerostomes. *Dog* urine, a single sample of which was tested, was more potent than human. Between 5 cc. and 10 cc. of fresh undiluted urine was necessary while 15 cc. of 1 : 1, 20 cc. of 1 : 2 and 25 cc. of 1 : 3 dilutions were similarly lethal. Sufficient pig and sheep urine was not available for critical tests but the few results showed that more than 15 cc. of pig and more than 10 cc. of sheep urine would be necessary to sterilize 40 grams of horse feces.

Mixed urine from a number of non-pregnant rabbits on a generous maintenance ration was available however, and a number of tests were carried out with it. The results showed that it had no killing power at all; in fact many of the tests gave higher numbers than the controls (Table II).

TABLE II

RABBIT URINE

Quantity of urine, cc.	Larvae recovered
1	50,000
2	59,000
3	32,000
4	39,000
5	69,000
7 5	41,000
10	38,000
15	15,500
20	41,000
25	14,000
Control xlv	34,000

Byre and Midden Fluid

During the summer of 1935, fluid was collected from a sump under a cow manure heap. This is referred to as "byre-fluid" and consisted of urine and water that was used to wash the floor and was carried out with the manure. This fluid was tested immediately upon collection. About 230 gallons were passed through about two tons of rotting stable manure and twice through fresher manure; this also was tested for lethal purposes. As a comparison with the last test and in addition to the usual controls, a similar quantity of water was poured over two tons of stable manure a few weeks old and allowed to stand a fortnight. The bottom fluid was then drawn off and tested; it is referred to as "manure water". All three tests are shown in Fig. 3. The byre-fluid had little killing properties, but the two fluids passed through manure were slightly more lethal. None was of practical value but the rather better results with the manure fluids suggested that the feces themselves might have some lethal property. There seemed a possibility that by reducing the surface tension of the fluid in a culture by adding soap solution, any lethal substances might have more ready ingress. Two soaps were used—a hard Castile soap (made from olive oil and soda) and a soft soap (made from cotton-seed oil and potash). The results however (Table III) gave no promise of this. Lucker (10) has shown that sclerostome eggs stored anaerobically in feces and water die slowly (although Gackstatter (7) believes that the development of the egg is merely retarded) and this may explain why the numbers collected from the manure fluids were less than those in the controls. The addition of water has the same effect (13) as have diluted manure fluids and diluted non-lethal urines.

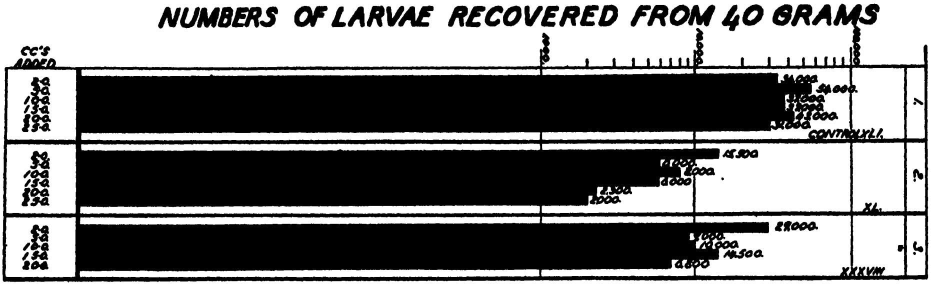

FIG. 3. *"Byre-fluid" and "manure water." Column 1 shows the effects of byre-fluid, column 2 of byre-fluid passed through manure; and column 3, of manure water.*

TABLE III

SOAPS

Amount of 1 : 4 solution, cc.	Number of larvae recovered	
	Hard soap	Soft soap
2.0	24,500	23,500
5.0	6,800	16,000
10.0	24,000	19,000
15.0	4,200.b.	9,500
20.0	900.b.	5,200
Control (See xvi Table IV)	25,000	25,000

There is a great variation in the chemical composition of urine, the variation depending not only on the species of animal but on the diet, age, or health of the individual. Apart from water, which may amount to as much as 95% of the whole, the main constituent is urea, which, while it may be almost absent, averages 1% to 4% in cattle (4) about 4% in horses, 2% in man (11) and up to 10% in dogs (15). In cattle, the urea tends to be lower in winter and to increase at calving or when cattle are put to spring grass. As synthetic urea is an important nitrogenous fertilizer, its effects on sclerostome larvae are discussed in the next paper in this series dealing with that group. The various salts of ammonia which occur in urine—chloride, phosphate and

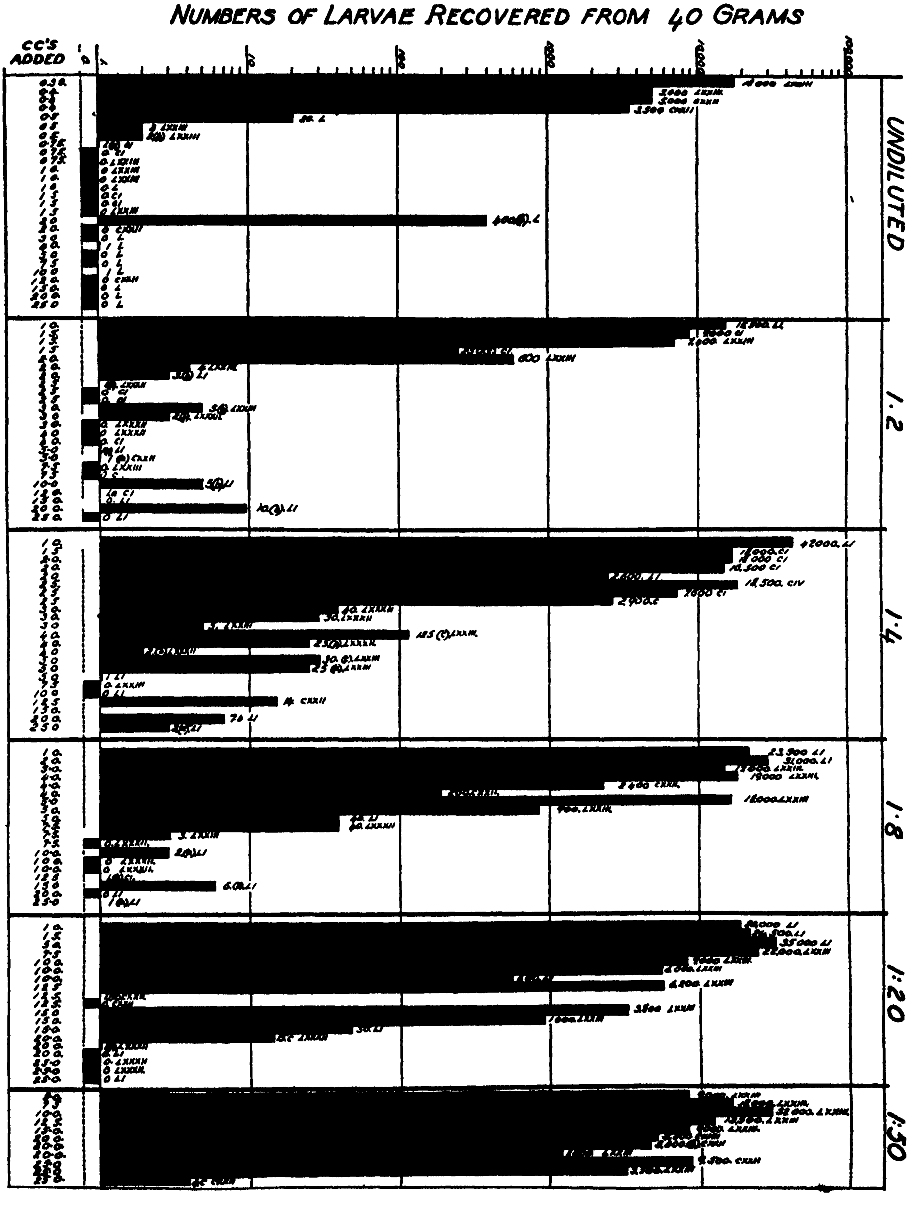

FIG. 4. *Ammonia water. The effects of varying quantities of various dilutions of ammonia water on horse feces.*

sulphate—are similarly used as artificial fertilizers and are manufactured synthetically; they are also discussed in the next paper. Ammonia itself however, is considered below. A large number of other organic compounds are also excreted in urine—uric acid, hippuric acid, creatinine, benzoic acid, bile pigments, ethereal sulphates of phenol and so on—but no opportunity has been found to test them. Acetone however has been tested and is discussed below; it is usually associated with ill health after calving, although it may be present even in healthy, pregnant or lactating cows, especially at the end of winter feeding (1, 2).

Among the various inorganic salts present in urine are compounds of sodium, potassium, calcium, magnesium and iron. These compounds are usually

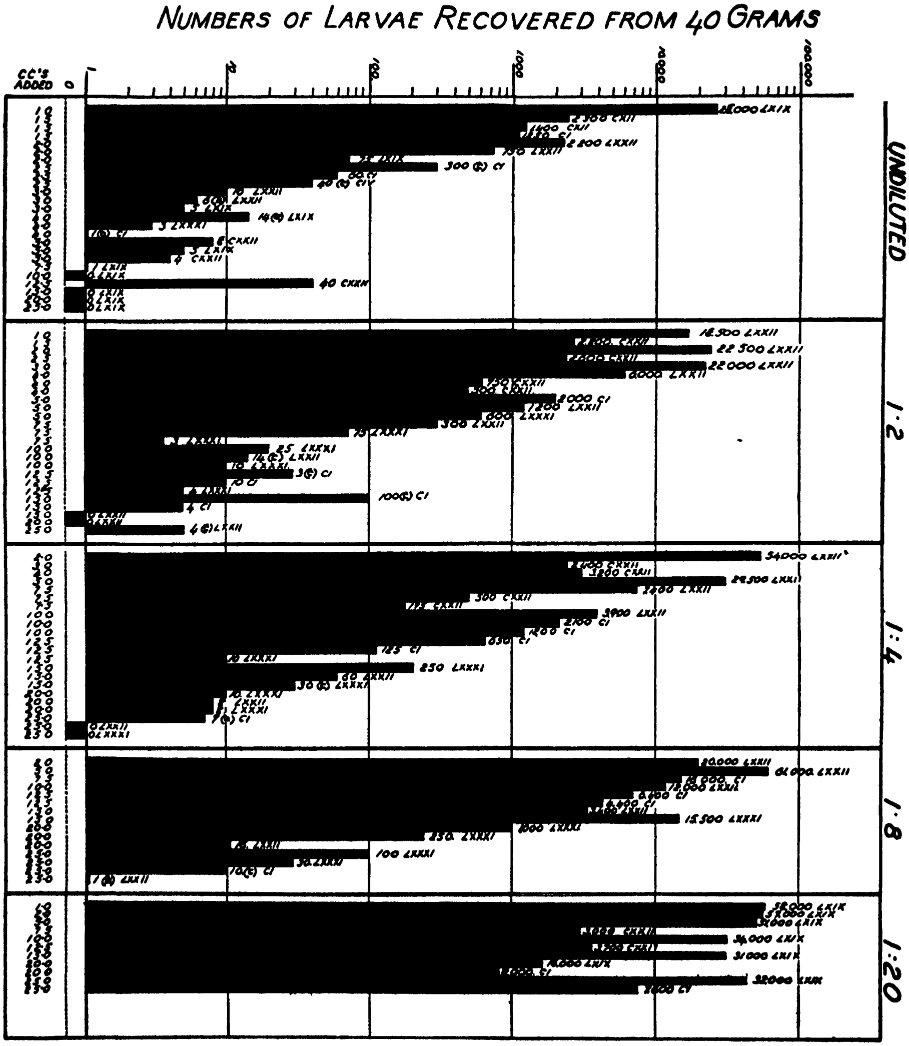

FIG. 5. *Acetone. The effects of various quantities and various dilutions of acetone on horse feces.*

found as chlorides, sulphates, phosphates and carbonates—and many of these have been tested. They will, however, be discussed in later papers in this series in connection with non-nitrogenous fertilizers or miscellaneous chemicals.

Ammonia was tested as ammonia water containing 27% NH_3. Experiments were made not only with various quantities but with varying dilutions made with water. The results of these are shown in Fig. 4. From these it is seen that as little as 0.75 cc. of undiluted ammonia water may prove lethal to all the larvae in a 40-gram sample. Diluted with twice its volume of water, 2.5 cc. is necessary, but with a 1 : 4 and higher dilutions, rather larger quantities (in terms of the contained ammonia gas) are necessary.

Acetone (or dimethyl ketone) is much less toxic than ammonia water. It also was tested in various quantities and varying dilutions (Fig. 5.). About 5 cc. of undiluted acetone are necessary to destroy all the larvae in a 40-gram sample; about three times this quantity of a 1 : 2 dilution and five times of a 1 : 4 dilution. Sufficient quantities of higher dilutions to prove lethal were not used. Acetone, accordingly, seems to differ somewhat from ammonia in its mode of action on the larvae. In the case of ammonia, more of the actual gas is necessary to kill, when employed in high dilutions, whereas with acetone, the amount seems constant for the three dilutions for which results are available.

TABLE IV

CONTROLS FOR TABLES I-III AND FIGS. 1-5

Series number	Date cultures made	Days kept in C.T. room	Average number of larvae isolated
xvi	22nd May, 1935	23	25,000
xxxviii	6th August	35	43,000
xxxix	9th October	20	35,000
xl	10th October	27	52,000
xli	10th October	28	65,000
xlv	31st October	45	34,000
xlviii	13th November	24	20,000
l	21st November	28	25,000
li	21st November	34	21,000
lxvii	7th January, 1936	16	67,000
lxviii	7th January	20	64,000
lxix	8th January	13	27,500
lxxii	22nd January	17	5,000
lxxiii	22nd January	20	14,500
lxxxi	13th February	24	28,000
ci	1st April	17	46,000
civ	7th April	23	21,500
cx	23rd April	20	10,500
cxi	24th April	21	17,500
cxii	24th April	24	7,800
cxxii	22nd May	17	4,400

This table shows the date of collection of the feces, the length of incubation and the numbers recovered. These data apply equally to the cultures controlled and recorded in Tables I–III and Figs. 1–5.

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THE BIOLOGY OF *IPS PERTURBATUS* EICHHOFF[1]

BY A. R. GOBEIL[2]

Abstract

The biology of *Ips perturbatus* in the Gaspé Peninsula is described from observations made during the summer of 1933 and 1934. This species generally breeds on trees killed by *Dendroctonus piceaperda* Hopk. but was also found on white spruce dying from the attacks of *Diprion polytomum* Hartig. In the Gaspé peninsula there is one complete generation a year. At the end of September and the beginning of October, the young adults leave their host tree to hibernate in the ground. Emergence from the ground occurs in the spring.

Experiments show that the adult stage lasts nearly two years. During their first complete summer as adults, the females lay two sets of eggs. The following spring they lay a third set and die during the summer. There are one to four egg tunnels per engraving. The average number of eggs per egg tunnel decreases with the increase in the number of females per engraving. Dissection of 500 beetles indicates that the two sexes are present in about equal proportions. Analysis of the results of a population study made on a white spruce representing 48 feet of tree superficies attacked by *Ips perturbatus* produced the following results: 2448 egg tunnels, 22,512 young beetles, 58,800 egg niches and a mortality rate of 58.33%.

Coeloides dendroctoni Cushm. is the most important parasite of this species, but does not kill more than 5% of the larvae and pupae. No trace of woodpecker control was noticed.

Introduction

Of all the injurious forest insects, the Scolytidae are among those which have been most thoroughly studied by entomologists. In spite of that, however, we know very little of the biology of these insects. Indeed, in America, apart from some species belonging to the genus *Dendroctonus*, which have been quite well worked out, the majority of the representatives of the other genera were considered more or less of secondary economic importance, and their study has been neglected. We cannot foretell, however, whether certain species actually considered as secondary might not eventually, owing to certain conditions of environment or for other reasons, become of great economic importance.

Ips perturbatus is one of the Scolytidae generally referred to as secondary bark beetles and, except for a few short papers published during the last ten years by Swaine (24) and Watson (26 and 27), all the information about it found in the literature is purely of a taxonomic character.

The distribution of this insect is very wide; it has been found almost everywhere in Canada and "apparently follows the northern range of its host tree" (23).

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This paper was presented at McGill University, Montreal, in partial fulfilment of the requirements for the degree of Master of Science.

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The observations recorded in this paper were made during 1933 and 1934 in the Gaspé Peninsula, province of Quebec. The work was carried on in the vicinity of the Federal Mine which is situated 1750 feet above sea level near the middle of the Peninsula, *i.e.*, 50 miles northwest of the village of Grand Cascapedia. The district is very hilly, and some of the highest mountains of the province occur within five to ten miles from the Mine. These are: Mount Lyall, 3000 feet; Mount Sterling, 3100 feet; Hogsback, 2725 feet; Mount Richardson, 3885 feet; and Mount Albert, 3775 feet. In this type of country the summer is rather short and precipitation is high. Hence the seasonal history of the insect in this region may be expected to differ from that on the coast or in other parts of Canada.

I. Host Tree

A number of attempts were made to breed *Ips perturbatus* on healthy white spruce, balsam and black spruce, but none were successful. In nature, this insect has never been found by the writer on dying black spruce, but under cage conditions the females cut egg tunnels, laid eggs, and the larvae lived all summer. It is doubtful, however, whether they could reach the adult stage before the advent of the first snowfall.

In 1933 and 1934, *Ips perturbatus* was found on white spruce that had been killed or much weakened generally by *Dendroctonus piceaperda* Hopk., or less often by *Diprion polytomum* Hartig. It enters the upper part of the trunk the year following the *Dendroctonus* attack. Thus, during the summer of 1934, *Ips* was found on trees that had been infested by *Dendroctonus* in June and July, 1933.

Although at the decline of a *Dendroctonus* outbreak, *Ips* is often as numerous as the *Dendroctonus*, it is not always found in the upper part of every tree killed by the latter. Indeed, if the latter species is present on more than half the total length of the trunk, the bark then seems to be too dry, and the portion of the trunk free from *Dendroctonus* is generally infested by *Dryocoetes affaber* Mannh. (see Table I, Trees 9, 12). In other cases, although the upper part of the tree presents suitable conditions for *Ips* infestation, examination reveals the presence of *Polygraphus rufipennis* Ky. only (Table I, Trees 7, 8, 10, 11). This is due to the fact that often *Polygraphus* enters the tree during the same year as *Dendroctonus*. Simpson (20) reports that, at Fredericton, N.B., *P. rufipennis* breeds three times during the same summer, in June, July and at the end of August. In the Gaspé, the number of broods of this species was not determined, but many adults were seen flying at the end of August. At that time of the year, it enters the trees infested by *Dendroctonus* in June of the same season, so that when *Ips* emerges the following spring, its place in many trees suitable for attack is already taken by *Polygraphus rufipennis*.

Twelve trees killed by *Dendroctonus* were cut, and the trunks were examined to gain a general idea of the distribution of secondary bark beetles on the different portions of the trunk and the type of trees preferred by each species, especially by *Ips perturbatus*. Table I shows the distribution of the different species of bark beetles in these trees.

The zones do not end abruptly; between zones there is an intermediate portion, one to three feet in length, in which the species of the two adjacent zones are intermixed.

Where *Ips perturbatus* and *Ips borealis* are found together on a portion of the trunk, as in Trees 1, 2, and 4, *I. perturbatus* is always the predominant species.

TABLE I

DISTRIBUTION OF SECONDARY BARK BEETLES ON THE TRUNK OF WHITE SPRUCE KILLED BY *Dendroctonus piceaperda*

Tree No.	D.B.H., in.	Total length, feet	*Dendr.* zone, feet	Other secondary bark beetles
1	13	61	28	*Ips pert.*, *Ips borealis* and *Dr. affaber* 14 ft., dia. 9–6 in.; *Ips borealis* only 24 ft., dia. 6–1 in.
2	8	48	16	*Ips pert.* and *I. borealis* 18 ft., dia. 6–4 in.; *Ips borealis* 12 ft., dia. 4–1 in.
3	12	72	22	*I. pert* 14 ft., dia. 8-6 in.; *I. borealis* 10 ft., dia. 3–1 in.; *P. rufipennis* 26 ft.
4	11	69	26	*I. pert* and *I. bor.* 28 ft., dia. 8–3 in.; *I. borealis* 13 ft., dia. 3–1 in.
5	12	67	27	*I. pert.* 22 ft., dia. 8–4 in.; *I. borealis* 16 ft., dia. 4–1 in.
6	9	66	13	*I. pert.* 32 ft., dia. 7–4 in.; *I. borealis* 18 ft. last 3 ft., *Pityophthorus* sp.
7	11	65	30	*Polygraphus rufipennis* 33 ft.
8	10	55	20	*Polygraphus rufipennis* 32 ft.
9	10	51	31	*Dryocoetes affaber* 18 ft. (tree very dry).
10	11	75	34	*Polygraphus rufipennis* 39 ft.
11	12	74	25	*Polygraphus rufipennis* 47 ft.
12	11	68	40	*Dryocoetes affaber* 25 ft.

Table I shows that *Ips perturbatus* generally attacks the portion of the trunk situated between the diameters of eight and four inches. In the upper part, its distribution is limited by the thinness of the bark. We might be inclined to believe that just the opposite condition limits their distribution on the lower part, but this is not the case; and if *Ips* is rarely found at the base of the trunk, it is because it generally goes to trees killed by ***Dendroctonus***, *i.e.*, trees whose bases are already infested when *Ips* enters them. But in some other cases, for example in trees almost completely defoliated by ***Diprion polytomum***, *Ips* is found at the base of the trunk. Thus on July 17, many *Ips perturbatus* tunnels were found at breast height on a tree of about 10 inches D. B. H. which, although much weakened by the saw-fly attacks, still bore green needles even on the lower branches. A more careful examination of the same tree, made on August 29, revealed the presence of *Ips perturbatus* for at least the first 40 feet of the trunk. Other secondary scolytids, such as *Ips borealis* Sw., *Dryocoetes affaber* Mannh. and *Trypodendron bivit-*

tatum Ky. were conjointly inhabiting the same portion of the trunk, but in much smaller numbers. On August 30, many other saw-fly-infested trees were examined and three of them were found severely infested by *Ips perturbatus* for the first 20 basal feet of the trunk. These trees were dead then, but they had certainly been alive in the spring, since it was still possible to see at the top of the tree the spring foliage which was, at the end of August, of a yellowish color.

We may conclude that in general *Ips perturbatus* follows *Dendroctonus* distribution, being noticeable one or two years after the appearance of *Dendroctonus*. When the *Dendroctonus* outbreak is at its peak, *Ips* has not attained its maximum numbers but does so when *Dendroctonus* is on its descending trend. By that time it is probably as numerous or even more numerous than *Dendroctonus*, but the decrease of the latter means also a decrease in the number of trees, on which *Ips* is able to live, so that its diminution will be as rapid as that of *Dendroctonus*. In Gaspé, however, a very interesting and particular case seems to occur. In 1934, while *Dendroctonus* was on its decline and *Ips* was probably at its maximum density, the number of trees infested by *Dendroctonus* not being sufficient for the *Ips*, many of the latter attacked white spruce severely defoliated by the saw-fly. As the trunks of these trees were absolutely free from *Dendroctonus*, *Ips* entered them at the very base and its population per tree was doubled. Therefore, it appears that in the absence of trees killed by *Dendroctonus*, those defoliated by the spruce saw-fly are in a condition suitable to *Ips perturbatus*. This case, however, is peculiar to the Gaspé Peninsula where two outbreaks, namely, of *Dendroctonus piceaperda* and of *Diprion polytomum*, exist concurrently. For this reason, it should be very interesting to follow the progress of *Ips perturbatus* in that particular district.

II. Bionomics

A. ADULT

1. Teneral Period

A tree, infested by *Ips perturbatus* in early June, was cut at the beginning of July and examined about twice a week by removing small pieces of bark. The first newly transformed adults were found on July 30, but they were few in number. On August 1 and 2, however, about 25% of the pupae had reached the adult stage. The young beetles fed very extensively; they obtained their food solely from the fibre of the bark, changing it to a kind of brown dust and leaving the larval mines and pupal cells almost unrecognizable.

On August 4, a log three feet long obtained from the tree under observation, was placed two feet above the ground, and a mat surrounded by "tanglefoot" was placed underneath to catch the beetles as they emerged. It was thought then that they would feed for a month or so and, consequently, would leave their host by the beginning of September. However, they did not leave the log until September 22. This seems to show that, around Kelly's camp, the young adults feed for about seven weeks before they emerge from their host. Of course, this statement applies only to the young beetles that reach the adult stage early in August. If the pupae transform to adults only at the beginning of September, as some do, they cannot feed much more than a month before the advent of the first snow, at which time they leave their host; these younger beetles can be recognized in the following spring by their paler brown color.

Occasionally some pupae do not reach the adult stage until the middle or end of September. In this case, the young beetles are still very immature at the time of the first snows and, as they are generally unable to hibernate in the tree, they die during the winter. Some observers have expressed the belief that the young adults could emerge from their host towards the end of July, and that there were two generations a year or at least one and a partial second, but the above study indicates that such is not the case in the Gaspé Peninsula.

2. *Emergence from Host-tree and Hibernation*

Many Scolytidae, such as *Dendroctonus* and *Dryocoetes*, pass the winter as larvae or adults in their host tree under the bark. Others, such as *Ips perturbatus* and *I. borealis*, leave their host in the fall and hibernate on the ground in the moss. This habit, however, is not common to all the species of the genus *Ips;* thus *Ips longidens*, like *Dendroctonus*, hibernates in the larval or adult stage under the bark where it matured (3).

It was believed for a long time that *Ips perturbatus*, like many Scolytidae, passes the winter in the host tree. To the best of my knowledge, Watson (26) was the first and the only worker to give correct information on the hibernating place of this species. Working in Algoma district, Ontario, he found that they overwinter as adults, after leaving their tunnels in September and October to hide in the ground.

With few exceptions, the same habit obtains in the Gaspé. The larvae in general reach the adult stage between the first week of August and the beginning of September. As said before, the young beetles feed for 1½–2 months and then drill through the bark to make an orifice, or "exit hole" of the same diameter as that of the entrance hole bored a few months previously by the parent beetles. In winter, a tree which has been severely infested by *Ips perturbatus* during the summer, is completely free of living insects of this species. The young beetles, which have matured too late in the fall to leave their host before the fall of the first snow, are always killed by the low winter temperature and are found dead under the bark the following spring.

Early in the spring, large numbers of beetles can be found in the moss beneath the tree they had attacked during the previous year. Many beetles are also found in the detritus scattered on the ground, under the bark of fallen trees, in the crevices of old stumps or other obstacles which stop the insects in their fall before they reach the ground. Some are present even in old *Dendroctonus* tunnels at the base of the infested tree.

In general, it can be said that the beetles leave their host tree in the fall to hibernate in the ground. However, if a tree infested by *Ips perturbatus* is cut during the summer, although the majority of the adults will leave the tree in the fall, quite a number will hibernate in their host and emerge from it the following spring. This was observed last spring when several living adults were found under the bark of a tree attacked by *Ips perturbatus* in the previous year and cut during the same summer. The same phenomenon was also observed in another tree presenting exactly the same conditions. Concluding then that even in standing trees a few adults might succeed in wintering, I immediately cut two trees infested by *Ips* during the previous year. Not a single living beetle was found in either of them. It may be assumed, that in standing trees these insects cannot, at least in that particular district, resist the low temperatures of the open during the winter. It is probable for this reason that, when an infested tree lies on the ground, the adults which have not left the tree before the advent of the first snow succeed in hibernating in their host tree.

In order to secure exact information as to the time of exodus of the young beetles from their host, a large mat was nailed on a wooden frame (Fig. 1), constructed during the middle of the summer, at the base of a tree attacked by *Dendroctonus* and subsequently infested by *Ips perturbatus*. The frame formed a square 8 × 8 ft., and the small joists on which the mat of factory cotton was nailed were two feet above the ground. A band of tanglefoot two inches wide surrounded the mat, while another band was also placed on the mat around the base of the tree to prevent the beetles from climbing the trunk. A third band of tanglefoot surrounding the tree itself was placed

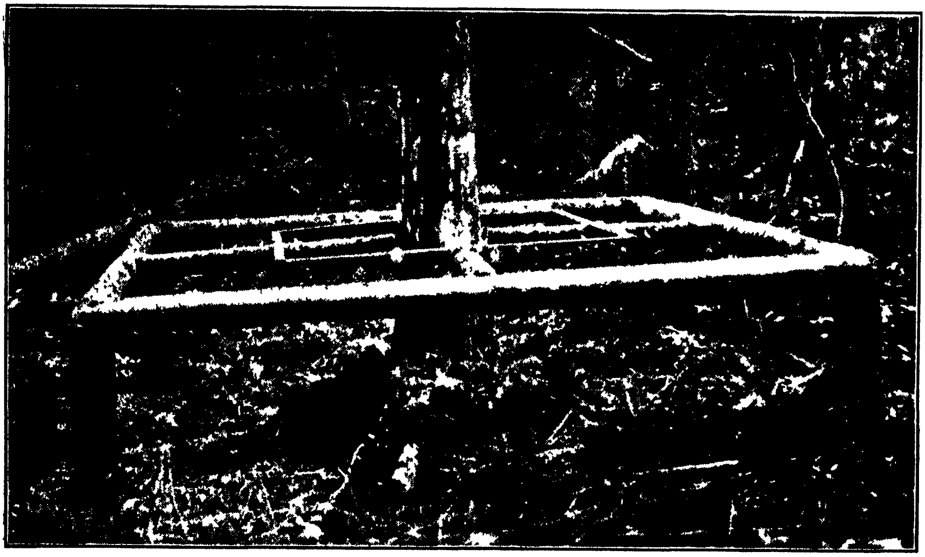

FIG. 1. *Wooden frame (on which a mat was nailed) used for records of Ips perturbatus emergence in the fall.*

one foot above the mat. This third band was used to determine whether the adults, when they leave their exit holes, simply fall from the tree or crawl down the trunk as some of Watson's observations in Ontario seemed to indicate.

This mat was constructed by August 23, and was examined at least twice a day, generally at 11.45 a.m. and 5 p.m. Table II shows the daily emergence from August 24 to September 28.

TABLE II

EMERGENCE PERIOD OF *Ips perturbatus* IN THE FALL

Date	Old parent beetles	Young adults	Date	Old parent beetles	Young adults	Date	Old parent beetles	Young adults
Aug. 24	26	—	Sept. 5	6	—	Sept. 17	1	—
Aug. 25	10	—	Sept 6	1	—	Sept. 18	—	—
Aug. 26	4	—	Sept. 7	2	—	Sept. 19	10	—
Aug. 27	5	—	Sept. 8	1	—	Sept. 20	3	1
Aug. 28	2	—	Sept. 9	—	—	Sept. 21	3	2
Aug. 29	1	—	Sept. 10	1	—	Sept. 22	—	—
Aug. 30	2	—	Sept. 11	—	—	Sept. 23	4	6
Aug. 31	7	—	Sept. 12	1	—	Sept. 24	1	6
Sept. 1	—	—	Sept. 13	1	—	Sept. 25	—	7
Sept. 2	1	—	Sept. 14	—	—	Sept. 26	2	15
Sept. 3	—	—	Sept. 15	—	—	Sept. 27	4	14
Sept. 4	14	—	Sept. 16	2	—			

The young adults began to abandon their host in appreciable numbers on September 23. However, the great majority probably emerged at the beginning of October. As I was forced to leave the district on September 28, the daily examinations had to be discontinued.

Although no young beetles left their host before September 20, the emergence records for each day are given from August 23 to show that there is, during the entire summer, a more or less continuous emergence of the old, black, parent beetles. At the end of August, however, these beetles did not go to new trees to lay more eggs as they do in July; they merely hid themselves from the light in the folds of the mat. The old beetles leave their tunnels before the young adults, simply because they have been feeding during the entire summer.

No beetles were found in the band of tanglefoot placed on the mat around the tree or in the one surrounding the trunk of the tree itself. It can therefore be assumed that the insect does not crawl down the trunk but simply lets itself drop to the ground. On leaning trees, however, it might be possible, as suggested by Watson, that the beetles crawl down the trunk from the exit hole.

The number of beetles which left the tree after September 20 is certainly greater than that indicated by the records. The action of the wind and the rebounding of the insect from one branch to another may cause it to fall much farther from the trunk than four feet. Nevertheless, the table gives a good idea of the beginning of the emergence and its rate of increase.

It was also discovered that these insects, although very small, could pass through a band of tanglefoot two inches wide after a few hours of struggle; this was the reason for inspecting the mat twice a day and sometimes oftener at the end of September. The majority of the beetles were found in the folds formed at the corner of the mat. It is apparent, therefore, that in the construction of apparatus of this kind for the study of bark-beetles, a board two to three inches wide should be nailed around the mat. The beetles would be more easily caught in such a device since they would hide themselves under the board. A narrow band of tanglefoot on the board would increase the efficiency of the trap.

3. *Spring Emergence*

Ips perturbatus emerges in the spring as soon as the snow has disappeared and the ground is thawed.

Two emergence cages were constructed to follow the period of emergence. One of the two, Cage A, was built on the slope while the other, Cage B, was set in the valley. These cages (Fig. 2) measured 2 × 2 × 2 ft., and each was placed at the base of a tree. The cheesecloth covering was kept in place by tacks lightly driven into each corner post to facilitate its daily removal for the liberation of the beetles. As soon as the beetles come out of the ground, they are attracted by the light, go to the top of the cage, and gather in the folds of the cheesecloth, especially at the upper corners. They were collected late every afternoon.

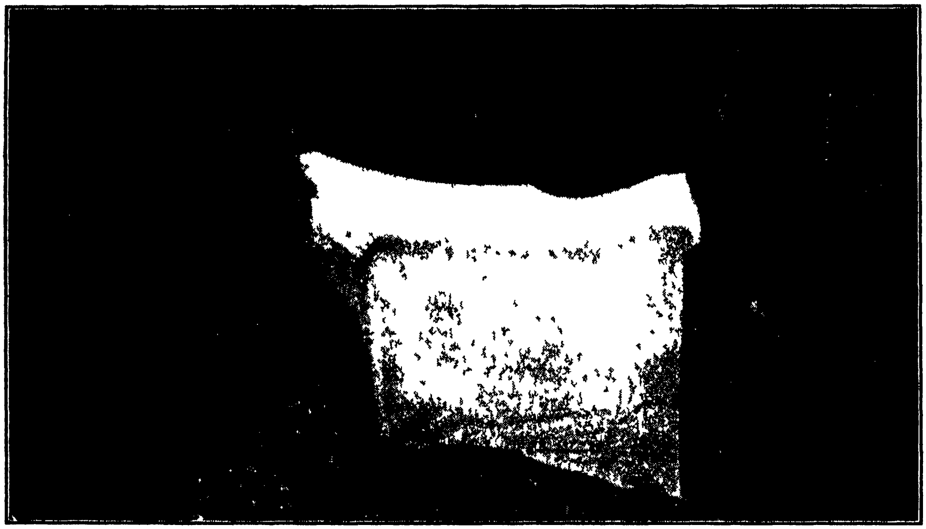

FIG. 2. *Spring emergence cage for Ips perturbatus.*

The results obtained from these cages seem to show that, other factors being equal, the beginning of the emergence period varies with the topography of the site, being earlier on the east slope than in the valley. Thus in Cage A, the first beetles emerged from the ground on June 3, while in the valley they were not observed until June 8, *i.e.*, five days later. It is quite possible that the daily period of insolation, being longer on the east slopes than in the valley, produces this accelerated or delayed emergence.

Table III shows the length of the emergence period in the valley and the number of *Ips perturbatus* collected every day in Cage B. The temperature and precipitation records for every day are also included, in order to show the relation between these two factors and emergence.

It can be seen from Table III that the emergence is intimately connected with the factor of precipitation. The temperature also influences the emergence period, but to estimate this influence properly, temperature records should also be taken under the moss within the cage. The second week of July was very warm, but at that time all the beetles had emerged from the ground, since none were found in the cage after July 11.

One may wonder whether the larvae that hatch from eggs laid by females emerging as late as July 11 will reach the adult stage early enough to leave their hosts before the advent of the first snow. This is dealt with in detail further on, but it can be said here that the progeny of parent beetles caged on July 9 reached the adult stage by September 7.

TABLE III

EMERGENCE PERIOD OF *Ips perturbatus* IN THE SPRING

Date	Emergence	Precipitation	Mean temperature at 4 ft. from the ground*	Date	Emergence	Precipitation	Mean temperature at 4 ft. from the ground*
June 8	44	—	43.25	June 25	—	0.04	55.30
June 9	—	Trace	43.25	June 26	—	—	53.25
June 10	64	—	53.00	June 27	2	—	51.00
June 11	—	0.30	50.85	June 28	7	—	54.10
June 12	—	Rain	51.85	June 29	—	0.55	55.80
June 13	—	Rain	46.40	June 30	—	—	52.75
June 14	—	Rain	45.15	July 1	16	—	52.30
June 15	—	0.68	45.55	July 2	—	0.74	57.00
June 16	—	—	48.25	July 3	—	0.04	55.80
June 17	46	—	52.00	July 4	8	0.38	57.45
June 18	—	0.09	54.40	July 5	11	0.06	55.25
June 19	31	—	58.20	July 6	4	—	61.75
June 20	—	0.70	49.85	July 7	—	0.48	61.50
June 21	43	—	54.70	July 8	1	—	55.70
June 22	—	0.30	53.30	July 9	9	—	57.55
June 23	—	—	52.45	July 10	4	—	56.65
June 24	20	1.98	57.25	July 11	3	—	62.30

* *The mean temperature was obtained by taking the average between the maximum and minimum temperature for each day.*

4. *Entrance Hole and Entrance Tunnel*

Ips perturbatus tunnels are not as a rule mixed with those of *Dendroctonus* except on a small section of the trunk in the intermediate zone. Its entrance holes are easily differentiated from those of *Dendroctonus* by the well known resin tubes found around the entrance holes of *Dendroctonus* but never present around those of *Ips perturbatus*, since the latter attack the trees only when they are partly dry. Furthermore, the entrance holes of *Dendroctonus* are much larger than those of *Ips*, the former being 3.0 to 3.5 mm. in diameter while the latter measure only 2.0 mm. in diameter.

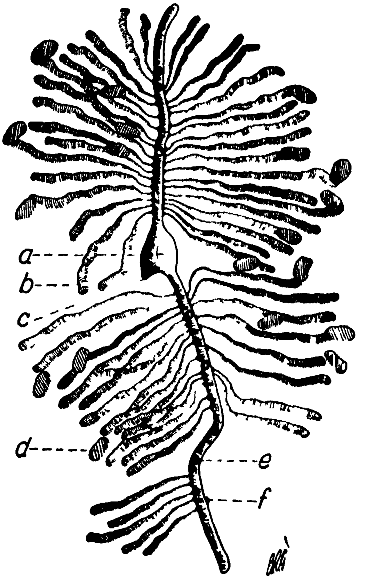

FIG. 3. *Ips perturbatus Eichhoff. General appearance of engraving with two egg tunnels. ½ natural size. (Original). a—nuptial chamber. b—larval mine. c—entrance tunnel. d—pupal cell. e—ventilation tunnel. f—egg niche.*

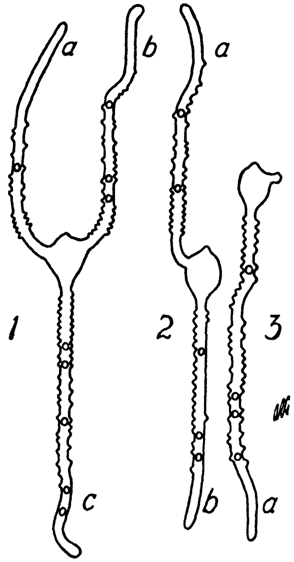

FIG. 4. *Ips perturbatus tunnels. (Original). ½ natural size. 1—Engraving with three egg tunnels, a, b, and c, (Triramous). 2—Engraving with two egg tunnels, a and b. Note that there are only three egg niches on the right side of tunnel b. Explanation on page 191 (Biramous). 3—Engraving with one egg tunnel, a (Uniramous).*

Quite often *Ips perturbatus* and *Ips borealis* share the same portion of the trunk. Their entrance holes are difficult to differentiate since they are almost of the same size. The entrance hole of *Ips borealis* has an average diameter of about 1.3 mm. while, as already said, that of *Ips perturbatus* measures 2.0 mm.

The entrance tunnel passes through the bark and leads to the nuptial chamber; its length varies with the thickness of the bark. On a standing tree these tunnels are always directed upward at an angle of 45 degrees. The diameter of the entrance tunnel, and of the egg gallery, is just large enough to admit the cylindrical body of the beetle. The entrance tunnels of *Ips perturbatus* are always cut by the males. Soon after the first beetles emerged last spring, a number of tunnels were opened, and wherever only one beetle was present it was removed and preserved in alcohol for sex determination at some later date. In 17 tunnels thus opened and found to contain only one adult in the nuptial chamber, all specimens proved to be males.

5. *Nuptial Chamber*

As stated in a previous chapter, the male cuts the entrance tunnel, excavates the nuptial chamber, and is then joined by from one to four females, each of which mines its own egg tunnel. The nuptial chamber is the central part of this subcortical dwelling place; all the passages formed by the egg galleries radiate from it. It is more or less triangular or sub-oval in shape and of quite constant size. The base of the isosceles triangle measures about 8 mm. and its height varies from 12 to 14 mm. The triangular shape is most striking in a triramous engraving, each egg gallery beginning at an angle of the geometrical figure (Fig. 4; 1). In a uniramous or biramous engraving, the nuptial chamber is more sub-oval in shape (Fig. 4; 2, 3). As Swaine (23) writes, the chamber is used for several purposes: "It serves as a temporary storage room for boring dust thrust into it by the females working in the egg-tunnels; it is also used by the beetles for turning or reversing their position, particularly by species which cut no ventilation-tunnels; and it is used regularly for copulation. With polygamous species the male spends nearly all his time in the nuptial chamber and in the entrance-tunnel".

6. *Excavation of the Egg Tunnel and Food Tunnel*

Several beetles were caged on June 10, and three days later when the cage was opened, the examination of the tunnels already started showed that one female had mined her egg tunnel to a length of 15 mm. and had excavated five egg niches on one side of it. This shows that as soon as the female has entered the nuptial chamber and copulated, she begins to cut the egg gallery and deposit eggs. This egg gallery is made partly in the wood and partly in the bark. In a biramous or triramous engraving, the egg tunnel opposite the entrance tunnel is generally cut by the first female to enter the nuptial chamber. Evidence of that is seen in uniramous engravings where the single egg tunnel is always situated opposite the entrance tunnel. This is also confirmed.by the fact that in polygamous engravings the egg gallery opposite the entrance tunnel frequently contains more eggs than the others, probably because these eggs are laid by a young female which is laying her first set and, consequently, has entered the tree just after emergence early in the summer.

When a female has laid her first batch of eggs, she needs a period of feeding before starting to cut a second egg tunnel. For that, she simply continues to mine and prolong the egg tunnel, which then takes the name of food tunnel or "food burrow".

Some experiments were undertaken to show the rate at which the females bore their egg tunnels, and at the same time to obtain information on the length of the egg stage. Many beetles were put in cages of the type illustrated; the tunnels cut by these beetles were examined every three days and the distance excavated was measured. Sixteen cages were constructed, each of them containing from 8 to 10 adults. In some engravings the beetles, being disturbed, left their tunnels; others were killed when the cages were opened. In many tunnels, however, I was able to keep track of the work done by the females for a period of more than a month. As expected there is a great variation in the distance excavated by the same female from one day to another, or by one female as compared with another. Some females mined only 13 to 14 mm. in three days, while others mined over 22 mm. in the the same period of time. Certain tunnels were left undisturbed for a month and when they were opened after that period, measured from 100 to 120 mm., while others had a total length of only 40 to 50 mm. These great variations, observed from one tunnel to another, are chiefly due to the different conditions found by the insect under the bark. But the daily variations in the length excavated by one female are mainly explained by differences in temperature, the insect being less active on cold days.

The information obtained on the egg stage is dealt with in another section of this paper.

7. *Egg Niches*

The female of *Ips perturbatus* deposits her eggs singly in small "niches" which she makes on each side of the egg tunnel. These niches, seen from above, have a triangular appearance, but from the side they appear cup-shaped. The lumen of the niche is of about 1.25 mm. long, while the depth varies from 1.0 to 1.25 mm. If we compare these proportions with the size of the egg, which is 1.06 mm. in length by 0.68 mm. in thickness, we find that the niche is much larger than the egg. This is necessary, however, since the egg is not simply deposited loosely in the niche, but is surrounded and packed in with dust by the female. This dust does not project into the egg tunnel, but simply fills the niche in such a way that the sides of the egg tunnels remain smooth.

Very often, especially at the beginning of the egg tunnel, there is only a short distance between adjacent niches. The niches are almost contiguous, and, as a matter of fact, I have counted as many as 20 egg niches in 1½ inch. Since the opening averages 1.25 mm., there is a space of 0.5 mm. or less between two consecutive niches.

A peculiar and very interesting case is found when two egg tunnels of different engravings are adjacent, being separated by less than ¼ inch. In this case, the female which is the last to start the egg tunnel (Fig. 4; 2b) does not cut any egg niches on the side adjacent to the other tunnel, *i.e.*, the right side in Fig. 4; 2b, probably because she is aware of the other female burrowing in the adjacent tunnel. Tunnels of that kind with all or most of the egg niches constructed on one side and none or merely a few here and there on the other side were often found.

Occasionally, although the egg tunnel may be quite long, the egg niches are not numerous and are widely separated one from the other. This may be due to the fact that the female which excavated this egg gallery was an old adult, which had already laid one or two sets of eggs before, and consequently had its power of reproduction much reduced. However, this subject requires further study.

8. Ventilation Tunnels

These are burrows placed at irregular intervals in the roof of the egg gallery and perpendicular to it. They may extend to or nearly to the outer surface of the bark or only halfway through it. There is no rule for their distribution and number; sometimes in a long egg gallery of three to four inches only one or two are present, while in other short galleries four or five may be found very near one another (Fig. 4; 1, 2, 3).

There are different opinions on the purpose of these tunnels which are present in the egg galleries of many species of Scolytidae. Some authors believe that they serve only for the aeration of the egg tunnel. Barbey (1) agrees with Chewyreuv that they serve the purpose of the nuptial chamber and he calls them "encoches d'accouplement" or copulation niches. He writes that: "Chewyreuv, ayant observé des Scolytides et Hylesines mis en élevage sous vitres, a pu constater que ces cavités n'avaient d'autre but que de permettre au mâle de s'accoupler pendant le forage de la gallerie de ponte." Swaine (23) states that they are used as "turning niches" for the female while she lays her eggs and as storage places for boring dust. Hopkins (12) believes that they serve for ventilation of the gallery and as storage places. There is probably some truth in all these statements. They may serve for ventilation but not exclusively for that purpose, since some of them do not go through the bark. Furthermore, unlike the *Dendroctonus* galleries, which often contain a mixture of resin and dust packed in the egg tunnel, the *Ips* galleries are, as a rule, always kept clean and have good aeration from the entrance tunnel. It is also probable that the so-called ventilation tunnels may serve for copulation as claimed by Chewyreuv and Barbey or as turning niches while the female lays her eggs. But, if this be their only purpose, it is difficult to understand why some should be found in the food tunnels where no eggs are laid. Even in these food tunnels they do not always serve for ventilation or as an opening to eject dust since some of them end bluntly in the bark. There is probably something more to be known about these tunnels.

9. Fertility and Longevity

It seems to be quite a common habit for many scolytid females to excavate more than one egg tunnel in the same season. Swaine (24) states that apparently *Ips perturbatus* and *Ips borealis* Sw. cut two egg tunnels in the same summer. Watson (27) wrote that *Dendroctonus piceaperda* (Hopk. lays two sets of eggs in the same season. Simpson (20) records *Polygraphus rufipennis* Ky. as cutting three egg tunnels in the same year and a fourth one in the following spring before death. He also found (21) that the female *Dendroctonus simplex* Lec. lays three sets of eggs during the same season. De Leon and Bedard and Terrell (8) working on *Dendroctonus monticolae* Hopk. found that two egg tunnels are cut by the females during the same summer.

To gather further information on that point in so far as *Ips perturbatus* Eichh. is concerned, a log of 12 inches in diameter and 3 feet long, heavily infested by *Ips perturbatus* on June 4 and 5, was caged on July 5 with another log 7 inches in diameter and 39 inches long, obtained from a tree blown down by the wind the previous winter and free of any kind of bark-beetle attack. At that time (the beginning of July), the beetles were well established in the infested log and some eggs had already hatched. These two logs when peeled on September 5, revealed 42 egg tunnels in the fresh stick while the original infested log contained 106 egg tunnels. The result indicates then that 40% of the females left the original stick and entered the fresh one to lay a second set of eggs. In reality the percentage should have been much higher, since the infested log was not caged until a month after the original infestation and it is very probable that some females had already left the log when the latter was placed in the cage. Moreover, some females, after they had completed their first egg tunnel, might have come out and started to cut a second egg gallery on the same log instead of entering the fresh stick. Nevertheless, the experiment shows that a high proportion of the females, if not all of them, lay two sets of eggs in the same season.

Many old parent beetles—differentiated from the young by the black color—leave their host tree in the fall to pass a second winter in the ground. Thirty-six of these old black beetles were kept separately in the moss to determine whether they would die during the winter or emerge again in the following spring and lay more eggs. Of these beetles, 13 were living the following spring (1934). On June 4, they were placed in three holes, ½ inch square, made in the bark of a log of spruce obtained from the top of a tree attacked by *Dendroctonus* the previous year. Each hole was covered with celluloid fastened to the bark with adhesive tape. When the log was examined on August 9, two egg galleries were found, one containing eight egg niches and the other ten; the progeny at that time were pupae and full-grown larvae. All the old parent beetles were dead in the galleries. The reason why only two egg tunnels were excavated may be partly that only a small proportion of the surviving beetles were of the female sex.

The conclusions of these experiments are:

1. That the females of *Ips perturbatus* lay two sets of eggs during the same season.

2. That the reproductive power of the female is greatly reduced following the second period of hibernation.

3. That the adult stage in some instances lasts nearly two years, the old parent beetles hibernating in the ground the second year to lay a third set of eggs and dying during the summer.

10. Relation between Fecundity and Proportion of Sexes in the Engravings

If the bark of a tree infested by *Ips perturbatus* is removed, we find numerous nuptial chambers, each of them used as rallying place for two, three or even four females, each mining her own egg tunnel.

Between the middle of July and the beginning of August, two trees were peeled and studied to provide an exact idea of the proportion of the females in the different types of brood burrows and the average number of eggs laid per gallery for each type. Often the egg tunnels of two different engravings unite and are mixed over a part of their length. This occurs chiefly when the density of attack is great. All such engravings occurring on definite regions of the trunk were studied except those not distinctly separated from one another.

Table IV presents in summary the data derived from a careful study of 87 engravings. It can be seen from the figures presented in this table that *(a)* the biramous engravings are the most commonly found, *(b)* the average number of eggs per egg tunnel decreases with the number of females per engraving, *(c)* the number of eggs per egg tunnel averages, on the whole, approximately 32.

TABLE IV

RESULTS OF THE STUDY OF 87 ENGRAVINGS OF *Ips perturbatus*

Type of engraving	%	Average no. of eggs per engraving	Average no. of eggs per egg tunnel	Average length of the egg tunnel
(a) *Tree No. 1:—Tree examined between July 13 and 15. 37 engravings or 70 egg tunnels yielding a total of 2138 eggs*				
Uniramous engraving	40.5	37.7	37.7	2.92
Biramous engrav.	32.4	60.8	30.4	2.66
Triramous engrav.	24.3	86.4	28.8	2.66
Quadriramous engrav.	2.7	101.0	25.25	2.25
Average		58.68	32.80	2.75
(b) *Tree No. 2:—Tree examined between Aug. 6 and 10. 50 engravings or 95 egg tunnels yielding a total of 2614 eggs*				
Uniramous engraving	34.0	35.00	35.00	3.25
Biramous engrav.	46.0	62.40	31.20	3.01
Triramous engrav.	16.0	75.00	25.00	2.83
Quadriramous engrav.	4.0	75.48	18.87	2.20
Average		55.62	31.00	3.00
(c) *Total average:—87 engravings or 165 egg tunnels yielding a total of 4752 eggs*				
Uniramous engraving	36.8	36.2	36.2	
Biramous engrav.	40.2	61.8	30.9	
Triramous engrav.	19.6	79.8	26.6	
Quadriramous engrav.	3.4	86.4	21.6	
Average		57.0	31.8	

Tree No. 2 was studied two weeks later than Tree No. 1. During this period the females were feeding all the time, thus increasing the length of the egg tunnels. This explains why the average length of the egg tunnels is higher in the latter tree and also why the total average length was not included in part "c" of the table.

As just stated the highest average number of eggs per egg tunnel is found in the uniramous engraving; the average decreases as the number of females per engraving increases. The decrease in the fecundity is not, however, directly due to the proportion of the sexes in the brood burrows, since small numbers of eggs were sometimes found even in uniramous engravings. The reason is to be found in the fact that in brood burrows of two, three or four egg galleries, the probabilities are two to four times greater that one of the females will be excavating her second or even third egg tunnel, *i.e.*, that she will have her reproductive power greatly reduced (see page 194). We also know that the male, after he has constructed the nuptial chamber early in the spring, is soon joined by the females. In a uniramous engraving there is a greater chance that this first and single female, which joins the male at the beginning of the season, will be laying her first batch of eggs and will consequently be able to produce the maximum number of eggs. Table V shows that large numbers of eggs per egg tunnel were not limited to the uniramous engravings, although on the whole the females under monogamic conditions produce more eggs than under conditions of bigamy or polygamy.

TABLE V

NUMBER OF EGG NICHES PER EGG TUNNEL IN THE DIFFERENT TYPES OF ENGRAVINGS

Number of eggs per egg tunnel	Occurrence in the different types of engravings							
	Uniramous		Biramous		Triramous		Quadriramous	
	No.	%	No.	%	No.	%	No.	%
1 - 10	0	0	2	2.8	3	6.0	1	8.4
11 - 20	1	3.1	10	14.1	10	20.0	6	50.0
21 - 30	6	18.8	23	32.4	22	44.0	3	25.0
31 - 40	17	53.1	19	26.8	9	18.0	2	16.6
41 - 50	5	15.7	8	11.2	6	12.0	0	0
51 - 60	2	6.2	5	7.1	0	0	0	0
61 - 70	1	3.1	4	5.6	0	0	0	0

Sixty-nine is the greatest number of eggs observed in one egg tunnel. These were found in a biramous engraving. The smallest number was six eggs, found in a branch of a triramous engraving. Table V shows that the majority of the uniramous engravings contain between 31 and 40 eggs; the biramous 21–40, while the average for the triramous lies between 21 and 30, and for the quadriramous engravings between 11 and 20.

11. *Numerical Relation of Sexes*

From what has been observed in the brood burrows, there occur nearly two females for each male. However, the dissection of 500 young beetles, obtained from the same portion of a tree, revealed about an equal number of males and females, namely 242 females and 258 males, or 48% and 51.6%.

Blackman (2) working on *Pityogenes hopkinsi* Sw., explains this anomaly between the actual counts and what is found in the brood burrows by two factors:— *(a)* some males die bachelors in their nuptial chamber, not succeeding in attracting any females, *(b)* the male is preyed upon by predaceous birds and insects while he is making the entrance tunnel, or he may be washed from his burrow by beating rain. There is probably some truth in Blackman's statements, but these factors alone can certainly not explain the disproportion of nearly two females to each male found in the brood burrows of *Ips perturbatus.* It is also doubtful that they can explain the disproportion, which is sometimes even greater, in the brood burrows of all the polygamous species of bark-beetles. Although I have stripped the bark of many trees infested with *Ips perturbatus*, and examined hundreds of engravings, I cannot recall ever having found even a single burrow containing an unaccompanied male. However, in order to explain, even if only in part, the disproportion of 2 or 3 females to 1 male found in brood burrows of polygamous species the proportion of bachelor burrows should be quite high. With regard to the second factor, in so far as *Ips perturbatus* is concerned, the influence of predaceous birds and insects amounts to almost nothing; *Ips perturbatus* is usually found where *Dendroctonus* occurs, and the woodpeckers, which are the most effective predaceous birds, are much more busy working on the comparatively big and plump *Dendroctonus* larvae than on small *Ips* mining their entrance tunnel in the upper part of the tree.

It appears to me that this disporportion of the two sexes in the brood burrows of polygamous species of Scolytidae could be understood readily if we consider two facts common to many polygamous species: *(a)* that among polygamous scolytids, the entrance tunnel is excavated by the male, *(b)* that the female lays more than one set of eggs during the same season. Nusslin (16), Blackman (2), Escherich (11) and others have noted that among the polygamous species, the entrance tunnel is cut by the male. On the other hand, as stated previously (9), many workers have also observed that among certain species of Scolytidae the female cuts more than one egg tunnel during the same season. The observations made by the writer on *Ips perturbatus* in the Gaspé Peninsula show that these two statements hold for the species. From all these observations we may legitimately conclude the following: The male digs the entrance tunnel and is soon joined by the female who cuts the egg tunnel and lays one complement of eggs. When the first egg tunnel is completed, this female feeds for a time and then leaves that particular brood burrow to start a second egg tunnel elsewhere. She cannot, however, like *Dendroctonus piceaperda* or other monogamous species, cut her own entrance tunnel and she has to look for one already made by a male. It generally happens, then, that she finds the latter already established with one or two females, but apparently the male readily receives this new arrival.

Therefore, although the total number of males and females is about equal, *Ips perturbatus* practices polygamy because each female cuts two egg tunnels during the same season and must rely on the male for the excavation of an entrance tunnel. As the latter digs only one of these entrance tunnels at the beginning of the summer, we arrive at an approximation of two egg tunnels per engraving.

B. THE EGG

The length of the egg stage, like the length of all the following stages, varies with the temperature under which development occurs. The first beetles began to emerge, in 1934, on June 3 and 4. Some were noted on a log on June 4, but most of the tunnels opened at that time contained only one adult, always a male. On June 5, many females entered the tunnels where they started to lay their eggs on June 6 and 7. The tunnels on that particular log were examined every two days but, the first larvae were not found until June 24. Hence, the eggs laid at the beginning of June took from 17 to 18 days to hatch.

The period of oviposition lasts about a month and a half, from the beginning of June to the middle of July. The period of incubation for eggs laid in July was not observed but, the daily temperature in July being higher than in June, the period of incubation should not be more than two weeks.

C. THE LARVA

1. Larval Mines

The larvae excavate their mines in the inner layers of the bark only. They do not score the wood as do the females when they cut their egg tunnels. Each larva burrows its own mine and the mines are always distinctly separated one from the other. They are filled with a dark brown frass which the larvae leave behind as they progress in their work. When the bark is removed from the tree, this frass makes the larval mines very conspicuous and easy to follow. They are more or less perpendicular to the egg gallery for the greater part of their length but towards the end they are often directed either upwards or downwards.

At their base, the larval mines are almost linear, but they increase in width as the larva grows and when the latter is full-grown the mine is nearly 2.0 mm. wide (Fig. 3; b). There is a great variation in the length of the mines; some measure only 40 mm., while others are as long as 80 or 90 mm. They are generally longer when the density of the population is low; thus if only a few beetles are caged on a log, the tunnels may reach 90 mm., but if the tree is heavily infested, the larval mines are rarely longer than 60 mm.

2. Length of the Larval Stage

The appearance of the first larvae was observed on June 24, and since some pupae were found on July 21, we may conclude that the larval stage lasts about four weeks. As the temperature at the end of June is about the same as that of the end of August in the Gaspé Peninsula, the length of the larval stage does not vary much, whether the eggs be laid early or late in the summer.

D. THE PUPA

When full-grown, the larva makes the pupal chamber or pupal cell and then ceases to feed. This pupal cell, which is generally located at the end of the larval mine, is an oval cell with rounded ends (Fig. 3, d). It is constructed entirely in the bark and is about 3 mm. wide × 6 mm. long and 1.5 mm. deep.

The first pupae were noticed last summer on July 21. At that time many full-grown larvae, ready to pupate and easily recognized by their milky white color, were taken from a log and placed in a tin box between two moist pieces of bark. Four of these larvae pupated on July 25. They were then removed and transferred on another piece of bark to a pupal cell previously made by other larvae. That bark was also kept on wet moss in a tin box and the piece of wood, which was originally covered by the bark, was fitted exactly and placed on top; thus the pupae were kept as nearly as possible under natural conditions. One of these pupae reached the adult stage on August 2, and the others on the following day.

The same observations were repeated at the end of August on full-grown larvae, which had developed from eggs laid at the beginning of July. Six of these larvae pupated on August 23 and reached the adult stage as follows: one on September 5, four on September 7, and one on September 8.

These two observations made at different times during the summer show that at Kelly's camp in 1934 the pupal stage, at the end of July, lasted only eight to nine days, while by the end of August and the beginning of September, when the daily average temperature was much lower, the pupal stage was as long as 14 to 16 days.

E. COMPLETE DURATION OF THE POST-EMBRYONIC DEVELOPMENT

Many beetles were caged at different periods of the summer and left undisturbed for 1½ to 2 months. When the tunnels made by these beetles were examined at the end of this period, most of the young were only full-grown larvae or pupae. They were then allowed to complete their development to the adult stage. In this manner, exact data were obtained as to the length of time required for the complete developmental cycle.

On June 3, many beetles were observed entering a log lying on the side of the road. This log was well exposed to the action of the sun. On July 29, the first beetles to be seen that summer were found on that log, but they were not very numerous before the beginning of August.

The beetles obtained from the emergence cages on July 9 were caged on a tree blown down by the wind the previous winter. When their tunnels were opened a month and half later (Aug. 20), many full-grown larvae were found; these larvae pupated on August 23 and reached the adult stage on September 7.

These two experiments, with others not mentioned here, made at different times throughout the summer, show that the duration of the post-embryonic development is about the same, approximately two months, whether the eggs be laid at the beginning of either June or July. The length of the larval stage is also the same (four weeks) in both cases. The pupal stage, however, is much longer towards the end of the summer than earlier, being respectively 14 days and 9 days. Consequently, although no experiments were conducted for the late brood, the incubation period must be shorter at the beginning of July than in early June when the temperature is still quite low on certain days.

Table VI is a résumé which gives an approximate idea of the length of each stage in the early and late broods.

TABLE VI

BIOGRAPHICAL DATES ON THE EARLY AND LATE BROODS OF 1934 IN GASPÉ PENINSULA

Early brood:	
June 3 and 4	Males enter the tree.
June 5 and 6	Females lay their first eggs.
June 24	Appearance of the first stage larvae.
July 21	Few larvae have reached the pupal stage.
July 29 and 30	Larvae which pupated on July 21 reach the adult stage.
Late brood:	
July 9	Males and females caged on a tree.
July 10	Females caged on July 9 lay their first eggs.
July 25	Eggs hatch.
August 23	Appearance of the first pupae.
September 7	Young adults.

III. Population in a Single Tree and Mortality

The number of beetles breeding in an infested tree varies directly as the area of the tree harboring *Ips perturbatus*. If the latter, instead of being the only species occupying the upper part of the trunk, is mixed with *Ips borealis* Sw. and *Dryocoetes affaber* Mannh., its number may be reduced to a minimum, sometimes less than 1,000. However, in certain cases where the weakened condition of the tree is due not to *Dendroctonus* but to *Diprion polytomum*, *Ips perturbatus* may occupy the total area of the trunk and the number of larvae reaching maturity will probably be more than 50,000. This is, however, a case peculiar to the Gaspé Peninsula, where two outbreaks, the spruce saw-fly and *Dendroctonus piceaperda*, occur simultaneously. In general the portion of the trunk infested by *Ips perturbatus* varies between 15 and 30 feet and the number of beetles reaching maturity is 10,000 to 20,000.

The most exact manner of obtaining information on the population of a tree infested by *Ips perturbatus* would be to peel carefully the whole tree under observation and make an exact count of the beetles found under the bark. It is possible, however, to obtain quite precise data by stripping the bark only on certain sections of uniform length and uniformly spaced. The latter method was the one followed for this population study.

The tree used had a total length of 66 feet and a D. B. H. of nine inches. The distribution of the different species of bark-beetles under the bark of the trunk was, from the base to the top, as follows:

Dendroctonus zone: 13 feet long; diameter at the lower part, 9 inches; diameter at the upper part, 7 inches.

Ips perturbatus zone: 32 feet long; diameter at the lower part, 7 inches; diameter at the upper part, 4 inches; average circumference, 18 inches; total superficies, 48 feet.

Ips borealis zone: 18 feet long; diameter at the lower part, 4 inches; diameter at the upper part, 1 inch, *i.e.*, 3 feet from the top.

Pityophthorus sp. zone: the last three feet.

On the portion of the trunk infested by *Ips perturbatus*, three sections, A, B and C respresenting the lower, middle and upper part of the infested portion, were cut. Their dimensions were:

Section A: 2 feet long × 20 inches circumference; cut at 9 feet from the base of *Ips perturbatus* zone, *i.e.*, at 22 feet from the D. B. H.

Section B: 1 foot long × 16 inches circumference; cut at 17 feet from the base of *Ips perturbatus* zone, *i.e.*, at 30 feet from the D. B. H.

Section C: 2 feet long × 15 inches circumference; cut at 22 feet from the base of *Ips perturbatus* zone, *i.e.*, at 35 feet from the D. B. H.

These three sections being cut, the bark was carefully removed and an exact count of the egg tunnels, egg niches, larvae, pupae, young adults, parasites, etc., was made.

Among the different methods of analyzing bark-beetle populations, the one proposed by Golovjanko and later modified by Iljinsky seems to be the most suitable. These two quantitative methods of special population studies, with many others, are very well summarized in a paper by de Gryse (6).

Using, with few modifications, the system of symbols devised by Iljinsky, the analysis of the above population study shows the following interesting facts:

A. Number of egg tunnels per square foot = 50*

M. Number of egg niches per square foot = 1225

O. Number of young adults per square foot = 502

b. Density of attack $= \frac{A}{1 \text{ (square foot)}} = 50$

d. Average number of eggs per egg tunnel $= \frac{M}{A} = 24$

c. Average number of young beetles per egg tunnel $= \frac{O}{A} = 10$

f. Percentage of mortality $= \frac{100(d - c)}{d} = 58.33$

The high percentage mortality "f" is the most striking character of all these data. This high percentage is almost entirely due to the factor "b", density of attack. Golovjanko was also of that opinion when he stated in his conclusions: "A decrease in, 'b' is followed by (i) an increase in the number of larval tunnels; (ii) *an increase in the percentage of larvae successfully completing their development* . . . . ." Iljinsky also shows conclusively that the density of attack is in itself a factor affecting the favorableness of the environment.

There are two reasons why a high density of attack of *Ips perturbatus* reduces the progeny of the families to less than half the original number: (i) The scarcity of food; in fact, it would be quite impossible for the progeny of 50 females per square foot, laying a total of 1,225 eggs, thus giving an average of 9 larvae to the square inch, to reach maturity in such a restricted space. Some will necessarily die by starvation. (ii) The other reason for this high percentage of mortality lies in the fact that when the density of attack is high a condition is often found in which, the egg tunnels are very near one another, almost contiguous. In such a case, what evidently happens is that when the young larvae of the egg tunnel have fed until they reach the egg tunnel they try to go through it, but the hardened dust which is deposited in the egg niches on one and sometimes both sides of the second tunnel will prevent the young larvae from crossing the second egg tunnel and cause them to die at that point by starvation.

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* *All these figures are obtained from section B only, since this is the only one where all the egg niches were counted.*

All the data obtained from this population study and not indicated in the previous figures are summarized in Table VII. It can be seen readily that the estimates for this particular tree give a total number of 2,448 egg tunnels or beetle families and 58,800 eggs, out of which a total of only 22,512 larvae reached maturity. Owing to the large number of egg niches per square foot, those of Section B only were counted and from this section the figures giving the percentage of mortality were obtained.

TABLE VII

Population study of *Ips perturbatus* on white spruce

Section	Living adults and pupae	Dead adults and pupae	Coeloides	Egg tunnels	Egg niches	Young adults per egg tunnel	Mortality, %
A. (2 ft. × 20 in. = 3⅓ sq ft.)	1732	104	75	189		9	
Average per sq. ft.	521	31	21	57			
B. (1 ft. × 16 in. = 1⅓ sq. ft.)	669	91	21	67	1633	10	58.33
Average per sq. ft.	502	68	16	50	1225		
C. (2 ft. × 15 in. = 2½ sq. ft.)	956	53	91	115		8	
Average per sq. ft.	384	21	36	46			
Total av. per sq. ft.	469	40	25	51		9	
Total popul. of the tree	22512	1920	1200	2448	58800		

It should be noticed that the average number of living pupae and adults is lower at the upper part of the trunk (Section C) than at the lower and middle parts, where we found the optimum conditions for *Ips perturbatus*. The thinner bark of the upper section makes the conditions less favorable for its development.

The item "dead adults and pupae" includes chiefly the pupae, since more than 50% of the progeny had reached the adult stage when the work was done. Moreover, most of the deaths were caused by *Coeloides dendroctoni* Cushman which generally kills the full-grown larvae and pupae. The number of dead pupae and adults is much larger in Section B than in the other two sections; the higher percentage of mortality in this case was caused by a white fungus surrounding the pupal cells and killing the pupae. This fungus however, was confined to only a small part of Section B.

IV. Parasites and Predators

As a general rule parasites do not play a role of great importance in the natural control of *Ips perturbatus*. And as can be seen by Table VII of the previous section, although *Coeloides dendroctoni* Cushm. is about the only and certainly the most important parasite of *Ips perturbatus*, the percentage of mortality which may be attributed to it is not over 5.3%. On some trees these parasites seemed to be concentrated more or less in spots, and under certain pieces of bark of about 6 ×10 inches, groups of 15 to 20 cocoons were found.

Coeloides dendroctoni is also probably the most important parasite of *Dendroctonus piceaperda;* it seems, however, to prefer *Ips perturbatus*, probably because *Ips* generally breeds in the upper part of the trunk where the bark is thinner and oviposition easier. This seems to be confirmed by the fact that *Coeloides* is always more numerous on a *Dendroctonus* infested tree with rather thin bark. Thus these parasites are generally found in greater numbers on trees 9 to 12 inches in diameter than on trees 18 to 20 inches in diameter.

It should be mentioned that examples of *Coeloides dendroctoni* associated with *Ips perturbatus*, although identical with examples feeding on *Dendroctonus piceaperda*, are smaller than the latter in all stages of development (larva, pupa and adult), probably because the host is smaller.

Besides *Coeloides dendroctoni*, a few small chalcids, which had reached the adult stage when collected on August 29, were also found in the course of the population studies. These parasites were identified by Mr. G. S. Walley as belonging to the genus *Pachyceras*, family Pteromalidae. The species of this genus seem to be associated [illegible] many different bark-beetles; thus De Leon (7) records the species *P*[illegible] *optogastri* as a primary parasite on *Dendroctonus monticolae* Hopk. [illegible] ies found in the Gaspé Peninsula. however, is not common and seem[illegible] ery little importance as a pa[illegible] of *Ips perturbatus*.

Although some predaceous in[illegible] ttack *Ips perturbatus*, noticed by the writer.

Among the predaceous birds, [illegible] ckers, which are so effect[illegible] *Dendroctonus*, are of very smal[illegible] nce as control agents of *Ip*[illegible] *turbatus*. This is understood if [illegible] at the woodpeckers are chiefly active in winter when *Ips* is hidd[illegible] round and safe from their attacks. It is true that they could feed [illegible] *ps* in the summer time, but since *Ips perturbatus* is usually close[illegible] ted with *Dendroctonus piceaperda*, the woodpeckers prefer to feed [illegible] atter species. However, since *Ips perturbatus* can breed on trees he[illegible] foliated by saw-fly and reach outbreak proportions even in the abse[illegible] *octonus*, the woodpeckers may in such circumstances exercise a[illegible] control. But actually such control, if any, must be very limit[illegible] no trace of woodpecker work has been found when the trees were cut [illegible] ll.

Therefore it may be asserted tha[illegible] es and predators are of secondary importance as control factors o[illegible] *batus*. The only effective check on the increase of the species is the [illegible] of food, *i.e.*, the absence of trees possessing the degree of weakness and dryness required to render them suitable as breeding places. Consequently, the decrease of primary outbreaks caused by *Dendroctonus piceaperda* or by *Diprion polytomum* will be the only adequate factors in ending *Ips perturbatus* outbreaks.

Acknowledgment

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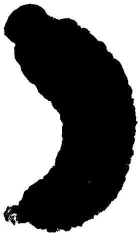
PLATE I



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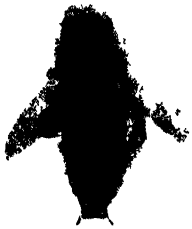

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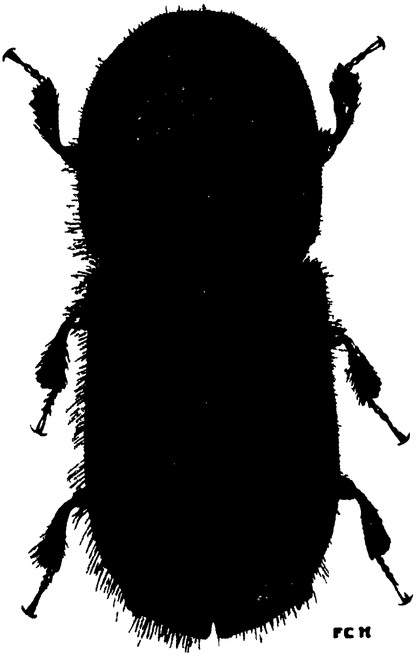

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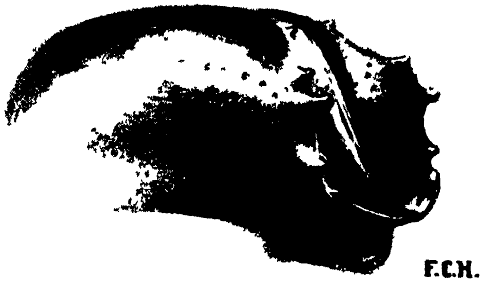

4

Ips perturbatus Eichhoff.

FIG. 1. *Larva. Magnified 7 dia (Original).* FIG 2 *Pupa Magnified 7 dia. (Original)* FIG. 3. *Dorsal view of the adult Magnified 20 dia. (After Swaine (24)).* FIG. 4. *Adult, showing the declivital teeth of the elytra Magnified 20 dia (After Swaine, (24)).*

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THE INTERRELATION OF BLOOD LIPIDS[1]

BY ELDON M. BOYD[2]

Abstract

The relation of the concentration of neutral fat, phospholipid, free cholesterol and cholesterol esters of blood plasma, the red blood cells and the white blood cells of man to increasing percentages of total lipid in each of these respectively was determined by oxidative micromethods in a series of more than 900 lipid extracts. A parallel increase in all four lipids was noted with increasing amount of total lipid in plasma until the total lipid exceeded the normal range. The earliest manifestation of a lipemia was a precipitous increase in plasma neutral fat and no increase or a decline in plasma cholesterol esters which later rose again as the lipemia developed. Increasing amounts of total lipid in the red blood cells was found due chiefly to phospholipid; although when the total lipid exceeded the normal range appreciable amounts of neutral fat appeared, the increase in cellular lipids bearing no direct relation to increases in plasma lipids. The same relation was established for the white blood cells as for the red blood cells but the leucocytes contained considerably more lipids than either plasma or the red blood cells.

A correlation has been found between the concentration of the several lipids present in blood in a number of situations. It is beyond the scope of this paper to discuss previous results where only two or three such lipids have been compared. The following is a brief review of previous typical studies in which all or nearly all of the known blood lipids have been considered. In 1921, Bloor (2) described the lipaemias of diabetes mellitus and of persistent hemorrhage as associated with increases in all of the plasma lipids. Similar approximately parallel changes in lipid percentages of plasma or serum were found by Bloor (3) and by Bender and Maynard (1) in relation to changes in diet; by Maynard, Harrison and McCay (26) and by Schaible (31) in relation to lactation; by Man and Peters (25) in relation to diabetic acidosis; by Stoesser and McQuarrie (34) and by Boyd (10) in relation to fever; by Page, Kirk and Van Slyke (30) and by Page and Farr (28) in relation to renal disease; by Boyd (9, 14) and by Boyd and Fellows (19) in relation to pregnancy; and by Boyd (11) in relation to the puerperium. In whole blood a parallelism was noted by Chaikoff, McGavack and Kaplan (23), but Okey and Boyden (27) failed to find one in their studies. Few attempts have been made to correlate changes in the red blood cells; Boyd (10) found a tendency towards parallel changes in fever. The author has examined the lipid content

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of the white blood cells in a number of conditions (6–8, 13, 16, 17, 20, 22) but has not hitherto recorded any possible relation of leucocytic lipid values to their total lipid.

The method used in the present work consisted in tabulating according to their total lipid values a large number of complete, differential lipid analyses of plasma, the red blood cells and the white blood cells of human blood. These samples of blood were from persons afflicted with a wide variety of diseases and from subjects who were found to have no condition known to affect the lipid values of blood. The numerous analyses were divided into groups of increasing total lipid values, the mean values of total lipid and component lipids were determined in each group, along with the standard deviations of the means, and an attempt was made to correlate the concentrations of the component lipids with that of the total lipid. In this manner it was possible to ascertain the average part played by each of the component lipids in the development of a lipaemia or of a lipopenia in plasma and to obtain some conception of the relative function of the lipids of the red cells and of the white cells in relation to their total lipid. Wide variations found in the values of certain of the cellular lipids rendered invalid definite conclusions based on comparisons of means. It may be noted that the term, *lipopenia*, was introduced by Boyd (10) to describe the occurrence of a general decrease in blood lipid values.

Oxalated or citrated blood was used and was taken usually from fasting subjects although it was not possible to insist upon fasting conditions in all cases. All of the subjects were on a standard, hospital diet, and under these conditions meals have been shown by Boyd (12) to have little influence upon the concentration of plasma lipids of normal persons although they may influence to some slight extent the lipid content of the red blood cells (21). It has been shown that oxalated, and presumably also citrated, plasma contains roughly 10% less lipid than heparinized or defibrinated plasma (18) while oxalated red blood cells contain proportionately more lipid than do red blood cells from defibrinated blood (15). Extracts were prepared and analyzed by Bloor oxidative micromethods as modified by Boyd (4, 5).

The Lipids of Plasma

The interrelation of blood lipid values will be considered under three main headings, the lipids of plasma, of the red blood cells and of the white blood cells. The interrelation in human blood plasma was determined from 421 complete, differential analyses representing over 4,000 lipid values. The series included 118 differential analyses on normal subjects, the remainder being of abnormal conditions. The lowest plasma total lipid was 100 mg. per cent (milligrams of total lipid per 100 cc. of plasma) and the highest was 1532 mg. per cent. The series was divided into 12 groups, Group 1 including cases with a plasma total lipid up to and including 199 mg. per cent, Group 2 from 200 to 299 mg. per cent, Group 3 from 300 to 399 mg. per cent, etc.

The Interrelation of All Cases

An analysis of these lipid values (approximately 4,000) has been presented statistically in Table I. In this and in succeeding tables and figures, the value of cholesterol ester has been calculated as the sum of ester cholesterol plus

TABLE I

THE LIPID COMPOSITION OF 421 SAMPLES OF HUMAN BLOOD PLASMA GROUPED ACCORDING TO THE VALUE FOR TOTAL LIPID OF EACH COMPLETE, DIFFERENTIAL ANALYSIS

(The lipid values are expressed in mg. per 100 cc. of blood plasma)

Group (Total lipid)	No. of cases	Value	Total lipid	Neutral fat	Phospholipid	Free cholesterol	Cholesterol ester
100–199	17	Minimum	100	13	21	5	13
		Maximum	185	91	90	29	52
		Mean	138	42	52	13	31
		Standard deviation	27	20	21	7	13
		Standard deviation in per cent of mean	20%	48%	40%	54%	42%
200–299	17	Minimum	200	0	28	11	18
		Maximum	298	201	139	39	114
		Mean	251	94	74	22	51
		Standard deviation	32	61	36	9	28
		Standard deviation in per cent of mean	13%	65%	48%	41%	55%
300–399	51	Minimum	303	0	42	6	2
		Maximum	398	283	191	42	185
		Mean	359	102	116	33	108
		Standard deviation	29	47	27	8	35
		Standard deviation in per cent of mean	8%	46%	23%	24%	33%
400–499	82	Minimum	404	28	52	28	0
		Maximum	499	270	200	90	245
		Mean	458	129	140	43	146
		Standard deviation	28	51	28	10	41
		Standard deviation in per cent of mean	6%	40%	20%	23%	28%
500–599	92	Minimum	502	0	105	23	97
		Maximum	599	300	235	65	299
		Mean	549	145	170	48	186
		Standard deviation	32	49	30	9	39
		Standard deviation in per cent of mean	6%	34%	18%	19%	21%
600–699	60	Minimum	602	11	113	27	65
		Maximum	695	437	259	93	419
		Mean	648	186	198	55	209
		Standard deviation	28	70	27	13	56
		Standard deviation in per cent of mean	4%	38%	14%	24%	27%
700–799	27	Minimum	703	90	156	45	66
		Maximum	798	408	285	100	272
		Mean	746	254	219	66	207
		Standard deviation	27	71	32	14	53
		Standard deviation in per cent of mean	4%	28%	15%	21%	26%

TABLE I—*Concluded*

THE LIPID COMPOSITION OF 421 SAMPLES OF HUMAN BLOOD PLASMA GROUPED ACCORDING TO THE VALUE FOR TOTAL LIPID OF EACH COMPLETE, DIFFERENTIAL ANALYSIS

(The lipid values are expressed in mg. per 100 cc. of blood plasma)

Group (Total lipid)	No. of cases	Value	Total lipid	Neutral fat	Phospholipid	Free cholesterol	Cholesterol ester
800–899	33	Minimum	801	97	151	47	0
		Maximum	896	528	422	136	367
		Mean	847	325	248	76	198
		Standard deviation	30	80	46	21	80
		Standard deviation in per cent of mean	4%	25%	19%	27%	40%
900–999	17	Minimum	901	242	173	60	99
		Maximum	997	570	314	92	389
		Mean	947	341	261	76	269
		Standard deviation	30	82	39	8	63
		Standard deviation in per cent of mean	3%	24%	15%	11%	23%
1,000-1,099	10	Minimum	1012	168	192	40	217
		Maximum	1082	518	352	108	569
		Mean	1053	384	292	81	296
		Standard deviation	24	103	42	18	97
		Standard deviation in per cent of mean	2%	27%	14%	22%	33%
1100–1299	6	Minimum	1103	230	268	95	130
		Maximum	1231	622	336	100	499
		Mean	1142	441	313	98	290
		Standard deviation	42	103	33	2	168
		Standard deviation in per cent of mean	4%	23%	11%	2%	58%
1300 and over	9	Minimum	1329	382	255	90	269
		Maximum	1532	773	510	123	481
		Mean	1401	540	393	107	361
		Standard deviation	65	116	75	10	68
		Standard deviation in per cent of mean	5%	21%	19%	9%	18%

cholesterol ester fatty acid as appeared in previous presentations of results (*e.g.*, 5). The method of determining and calculating the several lipids listed in these figures and tables has been previously described (5). In offering the results in this manner, the author has had in mind the simplification of description of data on blood lipids. As blood lipids are understood at present, there are four main groups; namely, the neutral fats, phospholipids, cholesterol esters and free cholesterol. Such values as ester cholesterol, total cholesterol, total fatty acids, etc., are convenient laboratory expressions since they represent values that may be determined by methods of analysis. When only part of a complete lipid analysis is performed, these laboratory values must needs be reported. When different methods of analysis are compared, it is also preferable to compare experimentally determined rather than calculated values. But for purposes of description such as is aimed at herein, these laboratory expressions may well be omitted. Such additional data may be calculated if desired, from the information given in the several tables, by applying the factors previously discussed (5).

The statistical analysis of results presented in Table I includes the listing of the minimal value for each lipid in each of the groups, the corresponding maximal value, mean value, standard deviation of the mean and, for purposes of comparing relative variations, the percentage standard deviation or the standard deviation expressed as a percentage of the mean. The standard deviation was calculated from the formula:

$$\varphi = \sqrt{\Sigma(x)^2/n}$$

where φ represents the standard deviation, Σ a summation symbol, x the difference between each individual value and the mean value and n the total number of values considered in the mean. To obtain the value of the standard deviation, each individual value was subtracted from the mean, these differences were squared, added together, divided by the total number of values and the square root of the resulting figure gave the standard deviation. From the mean and the standard deviation the expected range of values may be calculated. Thus two-thirds of values may be expected to lie within the range of the mean plus and the mean minus the standard deviation while 95% of all expected cases lie within the range of the mean plus and minus twice the standard deviation.

There was comparatively little variation in the values for *total lipid* in each of the groups given in Table I. The greatest relative variation occurred in the first group of 100–199 mg. per cent total lipid. In this group the mean was 138 mg. per cent and the standard deviation 27, which was 20% of the mean. The percentage standard deviation declined to 13% in the second group, to 8% in the third group, to 6% in the fourth group, and from then on it remained at low values between 2 and 5%. The percentage standard deviation for total lipid was lower in all except the first two groups than the percentage standard deviation of practically all the other lipids. The reason for this was that total lipid values were selected within a certain range and hence were not subject to the same chance variation as the other lipids. The standard deviations for the means were approximately the same in most of the total lipid groups, and since the means for total lipid became progressively greater, the percentage standard deviation obviously had to become progressively less.

Values for *neutral fat* were found to be amongst the most variable of all plasma lipids. The standard deviation may be seen from Table I to extend from 21 to 65% of the respective means. The neutral fat of human plasma was found to range from none at all to as high as 773 mg. per cent. In one group the range of values for neutral fat was from 0 to 201 mg. per cent although the total lipid figures of this group covered a range of only 98 mg. per cent. Neutral fat may enter plasma either from the tissues which store fat (neutral fat is the chief type of storage fat) or from the intestine during fat absorption. Part of it at least is carried to the liver where it is probably converted into phospholipid and cholesterol esters. During extensive parenchymatous damage to the liver, the author has noted a marked decrease in the phospholipid and cholesterol ester content of plasma with neutral fat values as great as, or greater than, normal, suggesting that in this condition the liver failed to convert the neutral fat coming to it into phospholipid and cholesterol esters.

The concentration of plasma *phospholipid* extended from 21 to 510 mg. per cent in the series of cases studied. There was less variation in the phospholipid content in the various groups than occurred in neutral fat. The standard deviations extended from 11 to 48% of the respective means, the greatest variation being again encountered in those groups with lowest mean values. One function of plasma phospholipid has been definitely shown by Sinclair (32) to be the transport of fatty acids. Boyd and Wilson (22) showed that phospholipids transport fat from the placenta to the human foetus *in utero*.

The *free cholesterol* content of plasma covered a range of from 5 to 136 mg. per cent. The standard deviation varied between 2 and 54% of the means, being greatest in the groups with lowest mean values. This fraction is usually designated "free" cholesterol to distinguish it from "ester" or "combined" cholesterol and from the sum of these, the "total" cholesterol. It would appear preferable to designate free cholesterol merely "cholesterol" and the other fraction "ester cholesterol" since the nomenclature in common usage has tended to imply that the only function of plasma cholesterol is its role in fat metabolism, and to render obscure the fact that cholesterol may, and probably does, have other important functions such as, for example, the possible relation of cholesterol to the genesis of sex hormones. At present, when merely blood cholesterol is stated, the inference is that blood total cholesterol is meant. Cholesterol is related to lipid metabolism in that it forms esters with fatty acids and it would appear, as previously stated, that this synthesis is effected at least in the liver. Sperry (33) has shown that there is in blood plasma a cholesterol esterase which splits cholesterol esters.

Values for plasma *cholesterol ester* were comparable in their variability to those of neutral fat. The concentration of this lipid extended from 0 to 569 mg. per cent and the standard deviation ranged from 18 to 58% of the respective means. Part at least of the variation in cholesterol ester values is due to the fact that this lipid is calculated from two or more experimentally determined figures, and hence its value would tend to contain a summation of experimental errors. Cholesterol ester is sometimes written in the singular, sometimes in the plural. While there is apparently only one fatty acid group attached to one molecule of cholesterol in the ester, there is little doubt but that in plasma there are many different compounds of cholesterol with various fatty acids.

The Relation of Component Lipids to Total Lipid of Plasma

In Fig. 1 the mean values of the individual lipids listed in Table I have been plotted against the mean values of the total lipid. The relation of one lipid to another in the lipopenic, normal or lipaemic phases may thus be clearly seen. This figure demonstrates that the three chief lipids of plasma are cholesterol esters, neutral fats and phospholipids and that the fourth so-called lipid, namely, free cholesterol, is present in much smaller concentrations throughout. In order to give some conception of the relation of these curves to the normal range of values, a "normal range" has been indicated in Fig. 1. This "normal range" is the expected range of 95% of values for total lipid of normal human adults as taken from Table III. The values of the several curves passing through the normal range in Fig. 1 are not necessarily normal values for these component lipids, since the curves are drawn from mean values of both normal and abnormal values. The relation of normal values for component lipids to their total lipid will be considered later.

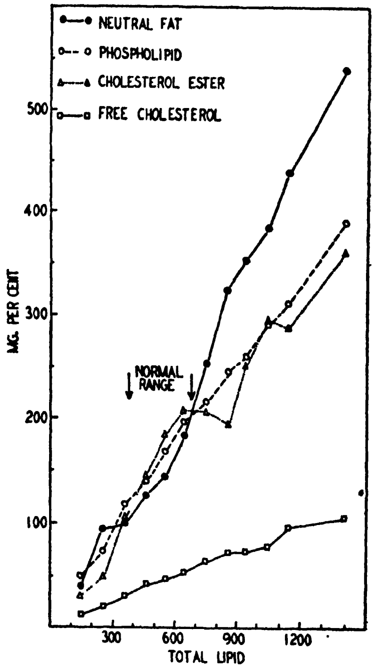

FIG. 1. *The relation of mean values of lipids in blood plasma of healthy and diseased persons to mean values of plasma total lipid expressed in mg. per 100 cc.*

Fig. 1 illustrates that the concentration of phospholipid and cholesterol ester follow an almost parallel course from the lowest lipopenic values to about the upper limit of the normal range of total lipid. Beyond this point, phospholipid continued to increase at much the same rate, bearing almost a direct linear relation to total lipid throughout. Cholesterol ester passed through a temporary declining or stationary phase when it reached the upper limit of the normal range, this phase persisting from average total lipid values of from about 600 to 900 mg. per cent. It may be inferred that the onset of a lipaemia is accompanied by a temporary decline in plasma cholesterol ester values. This peculiar behavior of cholesterol esters is emphasized because it has been recorded in the results of Gardner and Gainsborough (24) and, in somewhat modified form, in the results of Boyd and Fellows (19) in their studies of blood lipids in pregnancy of women and of guinea pigs. Such a phase in cholesterol ester values has not been stressed in previous considerations of the different types of lipaemia wherein it has apparently been missed by insufficiently detailed study. What its significance may be is at present obscure. It indicates that there must be some important difference in the metabolism of this lipid in contrast to the others and refutes the conception (26) that all lipid values of plasma increase at the same rate and at the same time. Following the phase of decline, the cholesterol ester content of plasma increased roughly parallel to that of phospholipid, with the exception of a further decline at total lipid values of about 1000 to 1100 mg. per cent. The second decline may be an artifact or it may indicate a tendency for fluctuation in plasma cholesterol ester values such as are found in hog cholera and in the lipopenia of fever in man (10), fluctuations which again have not been stressed in most other considerations of plasma cholesterol ester. It is apparent that much detailed study of the changes in the concentration of this lipid in particular and of all lipids in general is necessary before explanations as to their functions should even be attempted.

Values for free cholesterol exhibited, like those of phospholipid, a direct linear relation to total lipid. The similarity in behavior of these two lipids is further evidence in support of the hypothesis that they are closely allied in vital economy, the one being considered the antagonist of the other.

The Interrelation of Plasma Lipids in Normal Cases

In Table II have been listed values for plasma lipids in 118 samples of blood from normal human adults grouped according to the concentration of total lipid. The lowest figure for total lipid encountered in this series was

TABLE II

THE LIPID COMPOSITION OF 118 SAMPLES OF BLOOD PLASMA FROM NORMAL HUMAN ADULTS GROUPED ACCORDING TO THE VALUE FOR TOTAL LIPID OF EACH COMPLETE, DIFFERENTIAL LIPID ANALYSIS

(The lipid values are expressed in mg. per 100 cc. of plasma)

Group (Total lipid)	No. of cases	Value	Total lipid	Neutral fat	Phospholipid	Free cholesterol	Cholesterol ester
To 449	13	Minimum	381	28	98	32	102
		Maximum	447	118	186	56	188
		Mean	427	79	150	41	157
		Standard deviation	20	26	23	10	21
		Standard deviation in per cent of mean	5%	33%	15%	24%	13%
450–499	26	Minimum	451	50	97	32	109
		Maximum	499	188	200	62	245
		Mean	482	115	156	44	177
		Standard deviation	13	35	38	11	51
		Standard deviation in per cent of mean	3%	30%	24%	25%	29%
500–549	36	Minimum	502	55	111	23	104
		Maximum	546	223	212	64	219
		Mean	522	139	175	44	172
		Standard deviation	14	42	27	8	28
		Standard deviation in per cent of mean	3%	30%	15%	18%	16%
550–599	16	Minimum	551	85	105	35	132
		Maximum	593	213	210	63	284
		Mean	573	164	164	48	197
		Standard deviation	17	38	31	8	38
		Standard deviation in per cent of mean	3%	23%	19%	17%	19%
600–649	18	Minimum	602	125	113	27	65
		Maximum	645	437	228	88	254
		Mean	623	185	181	51	206
		Standard deviation	15	74	25	18	47
		Standard deviation in per cent of mean	2%	40%	14%	35%	23%
Over 649	9	Minimum	650	33	124	38	172
		Maximum	752	317	236	74	419
		Mean	683	202	187	53	241
		Standard deviation	34	81	33	13	70
		Standard deviation in per cent of mean	5%	40%	18%	25%	29%

381 mg. per cent and the highest 752 mg. per cent. The variation of total lipid values, as seen from the percentage standard deviation, was lower than that shown in Table I, which was to be expected since the results given in Table II were from a more homogeneous group of subjects. Plasma neutral fat extended from 28 to 437 mg. per cent and its standard deviation from 23 to 40% of the respective means, this lipid being the most variable in value of all lipids in the plasma of normal human adults. Phospholipid was the least variable of the component plasma lipids, the standard deviation ranging from 14 to 24% of the means. The free cholesterol values were almost as constant as those of phospholipid, the standard deviation extending from 17 to 35% of the respective means. Cholesterol esters extended from 102 to 415 mg. per cent and the standard deviations varied between 13 and 29% of the means.

For purposes of comparison with the preceding curves for both normal and abnormal lipid values in Fig. 1, a similar plotting of mean values against total lipid of normal subjects only has been offered in Fig. 2. It may be seen that the component lipid values of normal human plasma bore a relation to total lipid similar to that of all lipid values within the normal range of total lipid indicated in Fig. 1. The most notable differences were, first, a tendency for neutral fat to increase at a rate greater than that of the other lipids over the whole normal range of total lipid; and second, the concentration of cholesterol esters, although exhibiting fluctuations, did not enter the phase of decline as early as that seen in the plotting of both normal and abnormal cases in Fig. 1. These comparisons would suggest that with further study it may be possible to predict the imminent possibility of a lipaemia developing by a lipid analysis of plasma which may show a normal total lipid, a relatively high neutral fat and a normal or even low cholesterol ester, suggesting that a lipaemia is impending. The close parallelism between phospholipid and free cholesterol is even more evident in Fig. 2 than in Fig. 1. The data presented in Fig. 2 resemble a similar presentation of normal values by Page, Kirk, Lewis, Thompson and Van Slyke (29) in their Fig. 9. Their values for neutral fat are higher than those shown in Fig. 2 and Table II because their method of analysis gave higher values than the oxidative method.

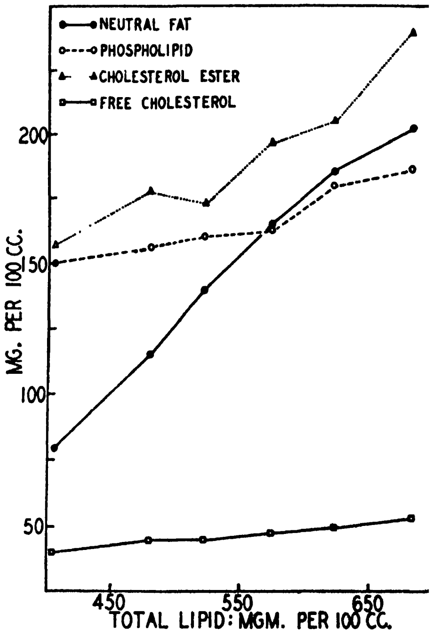

FIG. 2. *The relation of mean values of lipids in blood plasma of normal healthy human adults to mean values of total lipid expressed in mg. per 100 cc.*

The Normal Range of Plasma Lipids

The analyses of the plasma of healthy human adults have been considered as a whole in Table III in order to establish the normal range of lipids by oxidative micromethods based upon a large number of observations. These subjects were of both sexes, and a tabulation of results according to sex indicated that there were no significant differences between the lipid values of plasma of men and of women, providing that blood was not taken during menstruation, the climacteric, or pregnancy in women. The age of the subjects listed extended from 15 to 78 and within this range increasing age did not affect the concentration of plasma lipids. Below the age of 15 and especially in infancy and the newborn there was found a lipopenia but such results have not been included in the tabulation of normal values for healthy human adults. The author has previously reported normal plasma lipid values in occasional small groups of cases (5, 9, 12). These are included with many additional values in Table III. This larger group of normal cases revealed means similar to those previously found, except that the values were somewhat lower, especially for phospholipid.

TABLE III

THE LIPID COMPOSITION OF BLOOD PLASMA FROM 118 DIFFERENTIAL ANALYSES ON NORMAL HUMAN ADULTS

(The results are expressed in mg. per 100 cc. of plasma)

Value	Total lipid	Neutral fat	Phospholipid	Free cholesterol	Cholesterol ester
Minimum values*	381	28	97	23	65
Maximum values*	752	437	236	88	419
Mean values	530	142	165	46	177
Standard deviation	74	60	28	8	41
Standard deviation in per cent of mean, %	14	42	17	17	23
Expected range of 2/3 of normal subjects	456–604	82–202	137–193	38–54	136–218
Expected range of 95% of normal subjects	382–678	22–262	109–221	30–62	95–259

**The sum of the individual lipids does not equal the total lipid because the individual lipid values are taken from different analyses.*

The minimal and maximal values found for plasma *total lipid* were 381 and 752 mg. per cent. The extremes, which may be calculated to include 95% of all expected values, were 382 and 678 mg. per cent. The mean total lipid was 530 mg. per cent and the standard deviation of the mean was 74. In other words human plasma contains roughly from 0.4 to 0.7% of fatty substances or about the same as the amount of inorganic salts present and much more than the water soluble organic compounds such as the nitrogenous derivatives and glucose. In relation to amount present, the lipids of plasma are exceeded only by the albumins and globulins and are about equal in concentration to that of fibrinogen. The relation of this relatively large concentration of lipids to colloidal osmotic pressure of plasma and to the genesis of oedema has not received the study it should warrant, owing to its being in colloidal solution.

The total lipid of plasma was composed, on the average, of 33% of cholesterol esters, 31% of phospholipids, 27% of neutral fats and 9% of free cholesterol. The lipid most variable in amount may be seen from Table III to be neutral fat which yielded an expected range for 95% of expected values between 22 and 262 mg. per cent, a mean of 142 mg. per cent and a standard deviation of 60. The variability of cholesterol esters was smaller than that of neutral fat but greater than that of phospholipid and free cholesterol. The minimal value found for cholesterol esters was 65 and the maximal 419 mg. per cent, the mean 177 mg. per cent, the standard deviation 41 and the expected range of 95% of cases was calculated to be from 95 to 259 mg. per cent. Phospholipid and free cholesterol possessed the same relative variation, the standard deviations being 17% of each of the respective means.

The expected range of two-thirds and of 95% of normal subjects have both been included in Table III. The range of 95% of cases should be used when interpreting the significance of a single analysis, the range of two-thirds of cases when comparing a group of values with the normal. It is rare to find the expected range of two thirds of cases of any condition exhibiting a lipaemia or a lipopenia to be much beyond the expected range of two thirds of normal subjects. Any group of values not thus overlapping in its expected two thirds range the corresponding range of normal subjects may be considered to have demonstrated a significant difference from normal. Actually the correct statistical conclusion to be drawn in such a case is that the expected range of two thirds of all cases of the values in the condition under study may be considered to lie beyond the expected range of two thirds of normal values.

Lipids of the Red Blood Cells

The Interrelation of All Cases

A statistical analysis of the data obtained form 307 complete, differential lipid analyses of human red blood cells of normal and of pathological cases grouped according to the concentration of total lipid of each complete analysis has been presented in Table IV. These results have been condensed from

TABLE IV

THE LIPID COMPOSITION OF 307 SAMPLES OF HUMAN RED BLOOD CELLS GROUPED ACCORDING TO THE VALUE FOR TOTAL LIPID OF EACH COMPLETE, DIFFERENTIAL ANALYSIS

(The lipid values are expressed in mg. per 100 cc. of red blood cells)

Group (Total lipid)	No. of cases	Value	Total lipid	Neutral fat	Phospholipid	Free cholesterol	Cholesterol ester
300–399	7	Minimum	300	0	156	69	0
		Maximum	399	104	292	128	43
		Mean	354	40	209	94	11
		Standard deviation	51	39	46	22	15
		Standard deviation in per cent of mean	14%	98%	22%	23%	136%

TABLE IV—*Concluded*

THE LIPID COMPOSITION OF 307 SAMPLES OF HUMAN RED BLOOD CELLS GROUPED ACCORDING TO THE VALUE FOR TOTAL LIPID OF EACH COMPLETE, DIFFERENTIAL ANALYSIS—*Concluded*

(The lipid values are expressed in mg. per 100 cc. of red blood cells)

Group (Total lipid)	No. of cases	Value	Total lipid	Neutral fat	Phospholipid	Free cholesterol	Cholestero ester
400–449	13	Minimum	404	0	191	58	0
		Maximum	446	169	344	118	74
		Mean	431	26	296	93	16
		Standard deviation	13	46	40	19	27
		Standard deviation in per cent of mean	3%	177%	14%	20%	169%
450–499	65	Minimum	450	0	198	62	0
		Maximum	499	110	391	179	99
		Mean	477	38	301	106	32
		Standard deviation	16	35	38	18	24
		Standard deviation in per cent of mean	3%	92%	13%	17%	75%
500–549	63	Minimum	501	0	109	73	0
		Maximum	546	262	417	150	147
		Mean	527	62	313	111	41
		Standard deviation	22	48	51	19	34
		Standard deviation in per cent of mean	4%	77%	16%	17%	83%
550–599	49	Minimum	550	0	231	68	0
		Maximum	595	233	453	220	182
		Mean	573	52	353	128	40
		Standard deviation	14	54	50	28	40
		Standard deviation in per cent of mean	2%	104%	14%	22%	100%
600–649	25	Minimum	600	0	284	79	0
		Maximum	646	191	478	173	230
		Mean	627	64	384	126	53
		Standard deviation	14	59	34	22	27
		Standard deviation in per cent of mean	2%	92%	9%	17%	51%
650–699	23	Minimum	651	0	237	85	0
		Maximum	692	279	540	189	172
		Mean	670	63	430	136	41
		Standard deviation	14	78	68	23	53
		Standard deviation in per cent of mean	2%	124%	16%	17%	129%
700–799	21	Minimum	705	0	230	55	0
		Maximum	792	304	574	192	137
		Mean	743	153	422	138	30
		Standard deviation	28	101	84	33	48
		Standard deviation in per cent of mean	4%	66%	20%	24%	160%
800–999	21	Minimum	801	0	318	87	0
		Maximum	999	497	698	206	221
		Mean	882	216	467	141	58
		Standard deviation	39	142	89	27	61
		Standard deviation in per cent of mean	4%	66%	19%	19%	105%
Over 1000	18	Minimum	1000	0	272	50	0
		Maximum	1894	1380	864	217	391
		Mean	1266	557	502	117	90
		Standard deviation	298	426	170	43	116
		Standard deviation in per cent of mean	23%	77%	34%	37%	129%

more than 3,000 lipid values. The data were divided into 10 groups of increasing total lipid values as indicated in the first column of Table IV. Although presented in a manner similar to those of plasma, it should not thus be inferred that the lipid values of the red blood cells bore a direct relation to the lipid content of plasma. It should not be inferred, in other words, that as the lipid content of plasma increased, so necessarily did the lipid content of the red blood cells. As a matter of fact such a correlation was seldom found.

The *total lipid* content of the red blood cells varied between 300 and 1894 mg. per cent. As in plasma, the percentage standard deviation was greatest in the groups with lowest total lipid values, except in the last group which included a wide range of very high values. The standard deviation varied from 2% to 23% of the respective means for total lipid. The total lipid content of the red blood cells is approximately the same in amount as that of plasma, covering likewise a range of values similar to that of plasma. There is, however, a marked difference in the composition of the total lipid of the erythrocytes from that of plasma. The lipids being in colloidal solution or bound in the structural make-up of the cell, are not readily diffusible across the red cell membrane and changes frequently occur in the percentage of plasma lipids without being reflected by corresponding changes in those of the red blood cells. The correct degree of such changes is not evident when whole blood is selected for lipid analysis, as is unfortunately often done. There are comparatively few situations in which an analysis of whole blood for lipids can be justified as preferable to an analysis of plasma or of serum. In any thorough study both plasma and the red blood cells should be examined.

Phospholipid may be seen from Table IV to be the lipid of greatest bulk in the red blood cells, and it is believed that this phospholipid is composed chiefly of cephalins and sphingomyelins. The concentration of phospholipid in the several groups was relatively constant, the standard deviation varying from 9 to 34% of the means. It would seem that most of the phospholipid of the red blood cells is structural rather than metabolic in function. Boyd and Tweddell (21) found no diurnal variation in the phospholipid content of the red cells, and Sinclair (32) was unable to detect elaidic acid in the phospholipid fatty acids of the red blood cells of cats after feeding this special type of fat.

Free cholesterol is, next to phospholipid, the lipid of greatest bulk in the red blood cells. The values of free cholesterol were somewhat more variable than those of phospholipid but less variable than those of neutral fat and cholesterol esters. The standard deviation ranged from 17 to 37% of the means. Free cholesterol, like phospholipid, is believed to be largely structural rather than metabolic in function in the red blood cells.

Values for *neutral fat* (Table IV) may be seen to have been found extremely variable. In every group, no matter what the value of total lipid, at least one analysis was recorded in which the neutral fat content was zero. The maximum values in each of the groups ranged from 100 to 300 mg. per cent, except in the last two groups in which several cases with very high neutral fat values were encountered. The standard deviation extended from 66 to 177% of the means. It is obvious, therefore, that one should interpret with caution any relation between mean values for total lipid and mean values for neutral fat of the red blood cells. There is some evidence that the neutral fat of the red blood cells takes part in the lipid metabolism of the body (21).

The variations in the *cholesterol ester* content of the red blood cells were analogous to those of neutral fat (Table IV). In every group some analyses were found in which the cholesterol ester content was zero, although other members of the group had cholesterol ester values as high as from 43 to 391 mg. per cent. The standard deviation varied from 51 to 169% of the respective means. Thus one should again be cautious with cholesterol ester as with neutral fat in deciding upon any relation between mean values of this lipid and mean values of total lipid. Boyd and Tweddell (21) record evidence suggesting a diurnal variation in the cholesterol ester content of the red blood cells which would suggest that this lipid may also be concerned in sterol and lipid metabolism.

The Relation of Component Lipids to Total Lipid of the Red Blood Cells

In Fig. 3 the mean values for the component lipids in the several groups listed in Table IV have been plotted against the mean values of the total lipid. As in Fig. 1, the expected range of 95% of normal total lipid values has been indicated. The values of the component lipids as they pass through the normal range of total lipid shown in Fig. 3 are not necessarily normal values for these lipids. The normal values are shown in relation to total lipid in Fig. 4.

Fig. 3 clearly demonstrates that the two chief lipids of the red blood cells under normal conditions, namely, phospholipid and free cholesterol, bear a similar relation to total lipid. The rate of increase for phospholipid was greater than that of free cholesterol as the total lipid increased. Values for both lipids bore an approximately direct linear relation to total lipid values until the total lipid exceeded about 900 mg. per cent, after which the increase was less for phospholipid and the free cholesterol content actually showed a decrease.

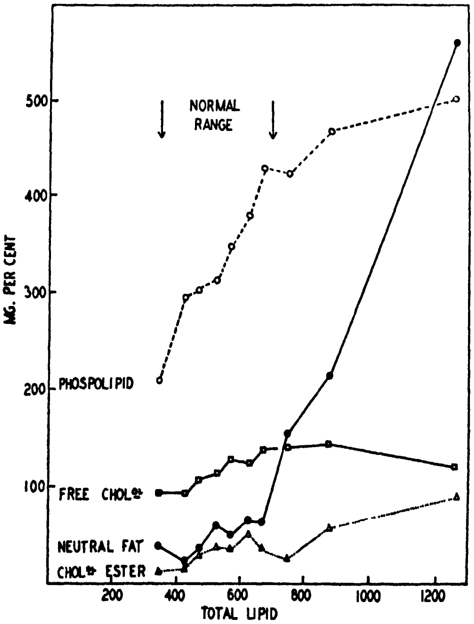

FIG. 3. *The relation of mean values of lipids in the red blood cells of healthy and diseased persons to mean values of total lipid expressed in mg. per 100 cc.*

The marked increase in neutral fat of the red blood cells beyond the upper limit of the normal range of total lipid is due, as is seen from the results in Table IV, to the occurrence of many exceptionally high values in the analyses within the high groups. At the same time other samples of erythrocytes were found to contain small amounts of neutral fat or none at all in these same groups in which very high values occurred. The obvious conclusion from Fig. 3 that the neutral fat content of the red cells is markedly increased when the total lipid content rises very high, cannot therefore be made without reservation. Rather should it be said that with very high total lipid values in the red blood cells one may meet with exceptionally high neutral fat values but that such need not necessarily be found.

A similar situation prevailed with cholesterol ester values. Within the limits of the normal range of total lipid the mean values of cholesterol ester were low but tended to increase with increase in total lipid. There was an interesting analogy between the curve for cholesterol ester in Fig. 3 and the corresponding curve of Fig. 1, in that at and just above the upper limit of the normal range of total lipid, the curve for cholesterol ester of the red cells declined temporarily as it did in plasma. In view of the wide variation in values averaged in the mean and as given in Table IV, it is not possible to attach more than coincidental significance to these similarities. The increase in the mean values of cholesterol esters above the normal range cannot be taken as unqualified evidence that cholesterol ester always increases with increase in total lipid because of the likewise marked variation that was found at this time. It may be stated that high values for cholesterol ester are often found, but not necessarily so, when the lipid content of the red blood cells rises above the normal range.

The Interrelation of Lipids of the Red Blood Cells in Normal Cases

Sixty-six samples of red blood cells from normal human adults were examined. The results obtained have been divided into six groups according to the total lipid values, and are presented in Table V. Variations in the

TABLE V

THE LIPID COMPOSITION OF 66 SAMPLES OF RED BLOOD CELLS FROM NORMAL HUMAN ADULTS GROUPED ACCORDING TO THE VALUE OF TOTAL LIPID OF EACH COMPLETE, DIFFERENTIAL ANALYSIS

(The lipid values are expressed in mg. per 100 cc. of red blood cells)

Group (Total lipid)	No. of cases	Value	Total lipid	Neutral fat	Phospholipid	Free cholesterol	Cholesterol ester
400–449	4	Minimum	425	0	239	90	12
		Maximum	442	71	302	103	66
		Mean	435	21	280	95	49
		Standard deviation	9	29	23	5	33
		Standard deviation in per cent of mean	2%	138%	8%	5%	67%
450–499	13	Minimum	454	0	243	79	0
		Maximum	493	110	293	116	99
		Mean	477	68	264	97	48
		Standard deviation	14	33	32	14	26
		Standard deviation in per cent of mean	3%	49%	12%	14%	54%

TABLE V—*Concluded*

THE LIPID COMPOSITION OF 66 SAMPLES OF RED BLOOD CELLS FROM NORMAL HUMAN ADULTS GROUPED ACCORDING TO THE VALUE OF TOTAL LIPID OF EACH COMPLETE, DIFFERENTIAL ANALYSIS—*Concluded*

(The lipid values are expressed in mg. per 100 cc. of red blood cells)

Group (Total lipid)	No. of cases	Value	Total lipid	Neutral fat	Phospholipid	Free cholesterol	Cholesterol ester
500–549	29	Minimum	501	0	220	73	0
		Maximum	549	144	334	138	112
		Mean	528	64	297	105	62
		Standard deviation	17	40	43	17	30
		Standard deviation in per cent of mean	3%	63%	14%	16%	48%
550–599	7	Minimum	552	11	231	68	0
		Maximum	597	167	437	134	110
		Mean	567	65	357	114	31
		Standard deviation	15	49	64	20	38
		Standard deviation in per cent of mean	3%	75%	18%	18%	123%
600–699	7	Minimum	634	54	237	129	0
		Maximum	692	279	418	189	55
		Mean	665	151	330	151	33
		Standard deviation	18	63	53	19	24
		Standard deviation in per cent of mean	3%	42%	16%	13%	73%
700–800	6	Minimum	709	13	274	144	0
		Maximum	789	302	452	192	135
		Mean	736	166	395	163	12
		Standard deviation	32	90	61	18	54
		Standard deviation in per cent of mean	4%	54%	15%	11%	450%

total lipid values were low in all groups. The standard deviation varied from 2 to 4% of the respective means for total lipid. The three lower groups contained zero values for neutral fat and in all groups the minimal value of this lipid was low. The standard deviation in the neutral fat values ranged from 42 to 138% of the respective means, a variation again sufficiently great to render dubious deductions regarding the relation of neutral fat to total lipid. The variations in the phospholipid values were much lower, the standard deviation extending from 8 to 18% of the means. The standard deviation represented 5 to 18% of the means for free cholesterol and hence mean values for both free cholesterol and for phospholipid may be taken as indicative of the trend of the values generally. An extremely wide range of values was recorded for cholesterol esters, the standard deviation being as phenomenally great as 450% of the mean in one group, showing that the means could hardly be regarded as indices for even a majority of values in a group.

With these variations in mind, Fig. 4 may be considered. In this figure the average values for the component lipids in each group have been compared with the means of the total lipid. Since the means for phospholipid and free cholesterol may be taken as accurate indices of the changes taking place in the majority of samples, it is apparent that phospholipid and free cholesterol parallel each other in changes in concentration which bear roughly a direct linear relation to changes in total lipid. One cannot add from Fig. 4 any further deduction regarding the relation of neutral fat and cholesterol ester to total lipid other than has been expressed above, because of the marked variation in the values averaged in the means. On comparing Fig. 3 with Fig. 4 it is evident that the values of the component red cell lipids of both normal and of abnormal cases follow the same general course when passing through the range of normal total lipid values.

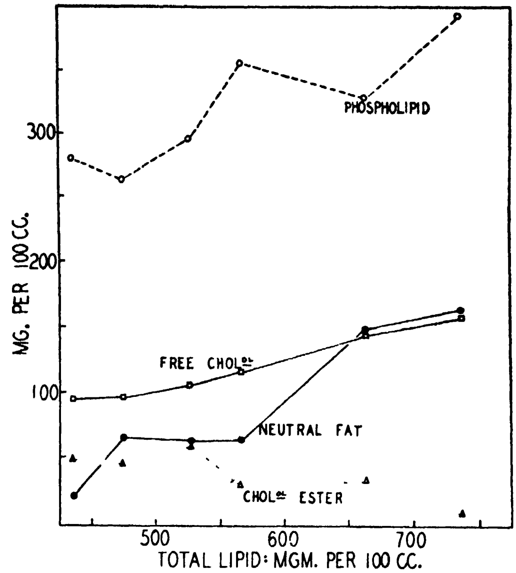

FIG. 4. *The relation of mean values of lipids in the red blood cells of normal healthy human adults to mean values of total lipid expressed in mg. per 100 cc.*

The Normal Range of Lipids in the Red Blood Cells

To establish the normal range of values for lipids in the red blood cells of healthy normal human adults, as estimated by oxidative micromethods, the results in Table VI are offered. The minimal total lipid value encountered in the 66 cases studied was 425 mg. per cent and the highest 789 mg. per cent

TABLE VI

THE LIPID COMPOSITION OF THE RED BLOOD CELLS IN 66 COMPLETE DIFFERENTIAL ANALYSES ON NORMAL HUMAN ADULTS

(The results are expressed in mg. per 100 cc. of red blood cells)

Value	Total lipid	Neutral fat	Phospholipid	Free cholesterol	Cholesterol ester
Minimum values*	425	0	220	68	0
Maximum values*	789	302	452	192	135
Mean values	523	84	311	115	13
Standard deviation	86	63	57	27	15
Standard deviation in per cent of mean, %	16	75	18	23	112
Expected range of 2/3 of normal subjects	437–609	21–147	254–368	88–142	0–28
Expected range of 95% of normal subjects	351–695	0–210	197–425	61–169	0–43

**The sum of the individual lipids does not equal the total lipid*because the individual lipid values are from different analyses.*

with a mean of 523 mg. per cent and a standard deviation of 86, which was 16% of the mean. The range of 95% of total lipid values for the red blood cells was practically identical to that found in plasma, *i.e.*, roughly from 0.4 to 0.7%.

Total lipid of the normal human red blood cells was composed, on the average, of 59.5% of phospholipid, 22% of free cholesterol, 16% of neutral fat and 2.5% of cholesterol esters. The lipid composition of the red blood cells is thus considerably different from that of plasma. The red cells contain more phospholipid and free cholesterol and less neutral fat and cholesterol esters than does plasma. The average value for red cell neutral fat was 84 mg. per cent, the standard deviation being 63 which was 75% of the mean, and the expected range of 95% of cases was from 0 to 210 mg. per cent. The expected range of 95% of cases for phospholipid was 197 to 425 mg. per cent, for free cholesterol 61 to 169 mg. per cent and for cholesterol ester 0 to 43 mg. per cent.

The Lipids of the White Blood Cells

The Interrelation of All Cases

In Table VII have been assembled the results of 174 complete, differential lipid analyses of human white blood cells, representing about 2,000 lipid values, grouped according to the concentration of total lipid. The lipid content of the white blood cells has been previously reported in a number of conditions (4, 6-8, 13, 16, 17, 20, 22) but has not previously been considered in relation to total lipid content. The results given in Table VII are expressed in mg. per 100 gm. of leucocytes, moist weight, and not in terms of mg. per 100 cc. of blood so that the lipid content of the leucocytes does not necessarily vary with the total leucocyte count.

There was little variation in the concentration of leucocytic total lipid, the standard deviations extending from 5 to 13% of the respective means of the several groups. Of the component lipids, phospholipid was most constant in value, the standard deviation being 19 to 43% of the means. The next least variation was in free cholesterol values, the percentage standard deviation varying from 21 to 38%. Neutral fat was quite variable with standard deviations extending from 63 to 125% of the means while cholesterol esters were most variable, the standard deviation ranging from 98 to 240% of the respective means.

Neutral fat and cholesterol ester have been shown to be the most variable of all lipids in each of plasma, the red blood cells and the white blood cells. A good deal of this variation is probably due to the fact that neither of these lipids are estimated directly but rather from two or more experimental figures. Cholesterol ester is calculated as the sum of ester cholesterol plus cholesterol ester fatty acid, which is calculated as 67% of ester cholesterol and this, in turn, is derived by subtracting the value of free cholesterol from that of total cholesterol, both of which are directly estimated. Neutral fats are calculated as 100/95 times the neutral fat fatty acids which are found by subtracting the values of the phospholipid and cholesterol ester fatty acids from that of

TABLE VII

THE LIPID COMPOSITION OF 174 SAMPLES OF WHITE BLOOD CELLS OF HUMAN BLOOD GROUPED ACCORDING TO THE VALUE OF THE TOTAL LIPID OF EACH COMPLETE, DIFFERENTIAL LIPID ANALYSIS

(The lipid values are expressed in mg. per 100 gm. of white blood cells, moist weight)

Group (Total lipid)	No. of cases	Value	Total lipid	Neutral fat	Phospholipid	Free cholesterol	Cholesterol ester
500–799	21	Minimum	501	0	257	82	0
		Maximum	798	244	552	220	139
		Mean	667	68	433	146	20
		Standard deviation	89	73	92	31	48
		Standard deviation in per cent of mean	13%	107%	21%	21%	240%
800–1099	32	Minimum	800	0	313	22	0
		Maximum	1081	488	780	259	468
		Mean	985	180	550	160	95
		Standard deviation	95	144	117	53	103
		Standard deviation in per cent of mean	10%	80%	21%	33%	108%
1100–1399	26	Minimum	1105	0	440	72	0
		Maximum	1392	642	1095	375	591
		Mean	1250	216	681	208	145
		Standard deviation	117	184	185	63	164
		Standard deviation in per cent of mean	9%	85	27	30%	113%
1400–1699	27	Minimum	1403	0	627	117	0
		Maximum	1679	638	1375	393	316
		Mean	1537	165	1030	257	85
		Standard deviation	105	207	195	64	107
		Standard deviation in per cent of mean	7%	125%	19%	25%	126%
1700–1999	21	Minimum	1726	0	269	63	0
		Maximum	1966	1004	1550	452	917
		Mean	1900	361	1030	271	238
		Standard deviation	95	308	300	98	232
		Standard deviation in per cent of mean	5%	85%	29%	36%	98%
2000–2399	22	Minimum	2015	0	411	129	0
		Maximum	2353	1390	1770	472	943
		Mean	2174	527	1218	302	127
		Standard deviation	118	330	323	84	226
		Standard deviation in per cent of mean	5%	63%	27%	28%	178%
2400–2799	11	Minimum	2423	0	823	245	0
		Maximum	2782	1460	2410	553	912
		Mean	2591	442	1527	399	223
		Standard deviation	128	476	432	109	291
		Standard deviation in per cent of mean	5%	108%	28%	27%	131%
Over 2800	14	Minimum	2586	0	354	171	0
		Maximum	3951	2650	2770	680	818
		Mean	3360	1156	1586	385	233
		Standard deviation	287	943	680	147	252
		Standard deviation in per cent of mean	9%	82%	43%	38%	108%

the total fatty acid. A summation of experimental errors must thus appear in the values of both neutral fat and cholesterol ester which may in a large part account for their variability.

In Fig. 5 the mean values of the component lipids have been plotted against the total lipid. Phospholipid and free cholesterol may be seen to bear a direct linear relation to total lipid as in the red blood cells, although the actual values of both lipids were much higher in the white than in the red blood cells. Considerable significance has been attached to the concentration of phospholipid in the white blood cells. The phospholipid content of a tissue appears to vary with its physiological activity, or tone, or functional capacity, or degree of training in the gymnastic sense. In conformity with this general hypothesis, Boyd (8, 13) has shown that increased values for phospholipid are found in the white blood cells of patients recovering normally from infection, while in patients who succumb to infection or combat it weakly, the phospholipid content of the white blood cells falls or remains low.

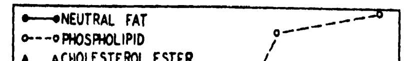

FIG. 5. *The relation of mean values of lipids in the white blood cells of healthy and diseased persons to mean values of total lipid expressed in mg. per 100 gm. moist weight.*

The increased amounts of neutral fat and cholesterol ester indicated by the rising mean curves in Fig. 5 cannot be taken as evidence that the values of these lipids bear a direct relation to the concentration of the total lipid. In Table VII it was shown that the standard deviation of the means for these lipids was relatively high. Hence it may be concluded only that frequently high values for cholesterol ester and for neutral fat appear when the total lipid content rises above the normal range.

The Interrelation of Lipids of the White Blood Cells in Normal Cases

The variations encountered amongst the lipid values of the leucocytes from all types of cases were greater than those seen amongst the normal cases as listed in Table VIII. The standard deviation of the total lipid values for normal cases extended from 4 to 14% of the means, of neutral fat values from 46 to 126% of the mean, of phospholipid values from 11 to 22% of the means, of free cholesterol values from 19 to 44% of the means and of cholesterol ester values from 54 to 144% of the means respectively. It is evident from Fig. 6 that the behavior of normal values for component lipids within the normal range of total lipid was analogous to that of all cases presented in Fig. 5.

TABLE VIII

THE LIPID COMPOSITION OF 30 SAMPLES OF WHITE BLOOD CELLS OF NORMAL HUMAN ADULTS GROUPED ACCORDING TO THE VALUE OF TOTAL LIPID OF EACH COMPLETE, DIFFERENTIAL ANALYSIS

(The lipid values are expressed in mg. per 100 gm. of white blood cells, moist weight)

Group (Total lipid)	No. of cases	Value	Total lipid	Neutral fat	Phospholipid	Free cholesterol	Cholesterol ester
To 1099	8	Minimum	688	38	442	76	0
		Maximum	1076	397	630	242	306
		Mean	935	118	578	162	77
		Standard deviation	130	105	62	50	111
		Standard deviation in per cent of mean	14%	89%	11%	31%	144%
1100–1399	8	Minimum	1128	0	457	72	14
		Maximum	1385	346	920	489	237
		Mean	1250	173	704	265	108
		Standard deviation	117	138	152	63	98
		Standard deviation in per cent of mean	9%	80%	22%	23%	91%
1400–1899	9	Minimum	1522	0	860	277	0
		Maximum	1894	595	1290	452	280
		Mean	1740	164	1111	326	139
		Standard deviation	142	206	125	142	108
		Standard deviation in per cent of mean	8%	126%	11%	44%	78%
Over 1900	5	Minimum	1963	61	990	245	0
		Maximum	2142	668	1380	420	155
		Mean	2040	486	1112	327	115
		Standard deviation	74	223	146	61	62
		Standard deviation in per cent of mean	4%	46%	13%	19%	54%

The Normal Range of Lipids in the White Blood Cells

A composite statistical presentation of results for leucocytic lipids of normal human adults has been given in Table IX. The total lipid content of normal human white blood cells was calculated to have an expected range including 95% of cases from 603 to 2291 mg. per cent or approximately three times as great as occurs in plasma and the red blood cells. On the average the total lipid of the white blood cells was composed of 60% of phospholipid, 16.5% of free cholesterol, 14.5% of neutral fat and 9% of cholesterol esters. It is apparent from Table IX that single values for leucocytic neutral fat may be

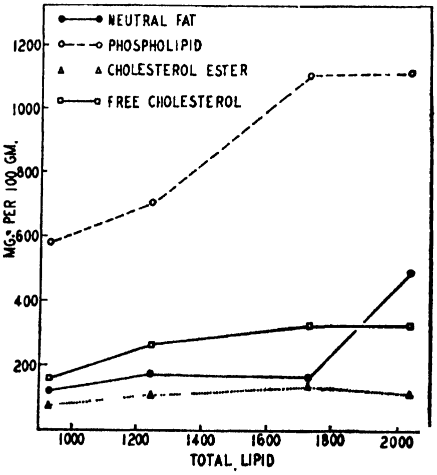

FIG. 6. *The relation of mean values of lipids in the white blood cells of normal healthy human adults to mean values of total lipid expressed in mg. per 100 gm. moist weight.*

TABLE IX

THE LIPID COMPOSITION OF THE WHITE BLOOD CELLS IN 30 COMPLETE DIFFERENTIAL ANALYSES ON NORMAL HUMAN ADULTS

(The results are expressed in mg. per 100 gm. of white blood cells, moist weight)

Value	Total lipid	Neutral fat	Phospholipid	Free cholesterol	Cholesterol ester
Minimum values*	688	0	442	72	0
Maximum values*	2142	668	1380	452	306
Mean values	1447	209	867	238	131
Standard deviation	422	212	281	95	121
Standard deviation in per cent of mean, %	29	101	32	40	92
Expected range of 2/3 of normal subjects	1025–1869	0–421	586–1148	143–333	10–252
Expected range of 95% of normal subjects	603–2291	0–633	305–1429	48–428	0–373

**The sum of the individual lipids does not equal the total lipid because the individual lipid values are from different analyses.*

considered normal if they occur within the range of from 0 to 633 mg. per cent. The corresponding expected range of 95% of values for phospholipid was from 307 to 1431 mg. per cent, for free cholesterol from 48 to 428 mg. per cent and for cholesterol ester from 0 to 363 mg. per cent.

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STUDIES ON THE ENDOPARASITIC FAUNA OF TRINIDAD MAMMALS

V. FURTHER PARASITES FROM THE OCELOT[1]

By Thomas W. M. Cameron[2]

Abstract

Several nematodes are recorded from the Ocelot *(Felis pardalis)*; and the whipworm, *Trichocephalus serratus*, is described in detail.

In the third paper of this series, the parasites of various carnivores including an ocelot (*Felis pardalis*), were described. Recently the entrails of a second ocelot from Trinidad were sent by Professor F. W. Urich to the Institute of Parasitology. The animal proved to contain one species of Acanthocephala and three species of nematodes, all in small numbers.

Echinopardalis pardalis (Westrumb, 1821) Travassos, 1918

One male and two females were recovered from the small intestine. The male measured 7.5 mm. and the females 8.5 mm. and 9.5 mm. respectively. The testes are elongated and situated one behind the other in the middle third of the body. The prostate glands are posterior to the second testis and are situated in pairs, although not so regularly as is described by Travassos (4). A very short neck is also present. These slight differences from Travassos' description however do not seem to be of specific importance, especially as the specimens are obviously young ones.

This acanthocephalid apparently is essentially a South American form and has been recorded from a number of Felidae (including the present host) on that continent. It has also been found in the domestic cat in North America.

The ocelot also contained a few examples of *Ancylostoma caninum* (Ercolani, 1859) and of *Molineus pardalis* Cameron, 1936, as well as several whipworms.

Trichocephalus serratus von Linstow, 1879

Two male, one immature and three mature female whipworms were found.

Both sexes are remarkable for their very small size and for the great length of the "neck". The mature females varied from 15.75 to 13.5 mm. in total length (av. 14.75) while the males were 12.5 and 13 mm. respectively. The average body length of the female was about 2.75 mm. and of the male, 3.4 mm. This gives a ratio of 5 : 1 for the length of "neck" to the body in the female and over 3 : 1 in the male. The females are 0.35 mm. wide and the males 0.3 mm. The head end in each sex is similar and typical. There

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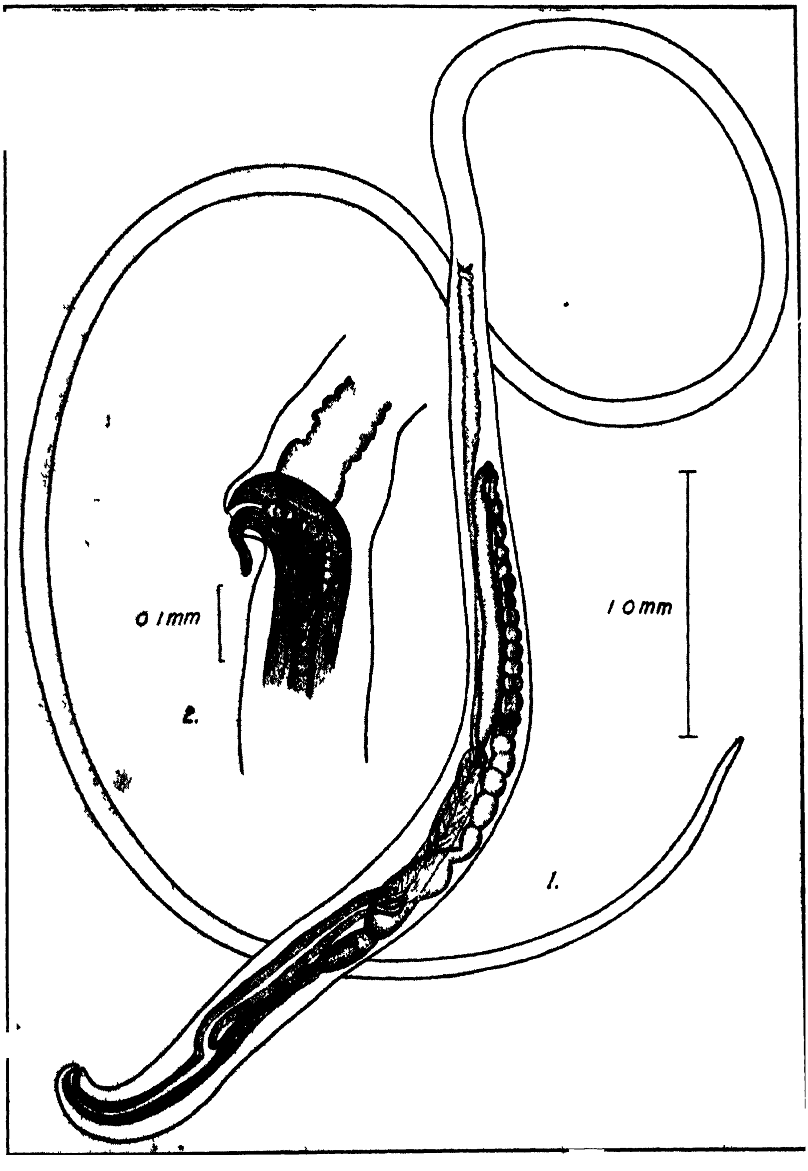
is a single broad, bacillary band present in the "neck", originating close to the head and terminating about the level of the body. The remaining part of the "neck" and all the body is finely striated transversely.

The female is a more or less typical whipworm, the vulva being situated at the junction of "neck" and "body". From the lower lip of the vulva



Trichocephalus serratus.

FIG. 1. *Entire male whipworm.* FIG. 2. *Vulvar region of female.*

however (Fig. 2) there projects a smooth finger-like process. The female genital system is typical. The ovarian tubule originates close to the anus and passing forward to the level of the vulva, turns back again to just anterior to its origin. There it turns and becomes a voluminous uterus which opens at the vulva. In the young specimen, this uterus appeared to be divided into two parts, but no such division was noticeable in the adults. In this same specimen, there were two pairs of minute sessile papillae just anterior to the anus. The eggs are of the typical whipworm type, show little variation in size, and measure (*in utero*) $60\mu \times 35\mu$.

The male (Fig. 1) has no terminal spiral and superficially resembles the female. Unfortunately in both cases the spicule was retracted but both specimens were well preserved and the details clearly visible. However, it is not possible to say whether or not the "sheath" is campanulate when extruded. It is spinose, and when retracted is about 0.4 mm. in length. The spicule, 1.2 mm. long, is simple and filiform. Its anterior end is attached to a large retractor muscle. About half way down the spicular canal, it is joined by a voluminous muscular tube which splits at its anterior end to join the rectum and male duct. This cloacal tube is presumably contracted towards the rear end of the body when the "sheath" is extruded. The male genital opening is dorsal to the rectum and a short canal joins the cloaca with a massive cement gland. This in turn communicates with the seminal vesicle by a narrow opening. The seminal vesicle runs as far as the junction of intestine and oesophagus where it communicates with the single testicle. This is a wide, laterally convoluted tube, which originates just posterior to the junction of the male and digestive tubes.

The digestive system is quite typical.

Whipworms are somewhat rare in Felidae and such records as exist all refer to South American cats. The sole reference to whipworms from this particular host is one by Canavan (2) who found a single female 42 mm. long in an ocelot which had died after a short period of captivity in the United States.

In 1851, Diesing recorded *T. felis* from *Felis tigrina* in Brazil but his species is not recognizable.

In 1879, von Linstow described *Trichocephalus serratus* from the domestic cat, and in 1889 he described *T. campanulus* from the same host; both examples came from Brazil. In 1923, Urioste examined some specimens from the cat from the same country and concluded a single species existed: this he called *Trichocephalus serratus*.

There are few details in this genus available for a morphological comparison of species and resource has to be made to the very unsatisfactory criterion of measurements.

	T. serratus (von Linstow)	*T. campanulus* (von Linstow)	*T. serratus* (Urioste)	Present species
Male	40 0 mm.	Not stated	20.5 mm.	12.75 mm.
Spicule	3.9 mm.	Not stated	1.5 mm.	1.2 mm.
Female	48.0 mm.	31 5 mm.	23.0 mm.	14.75 mm.
Eggs	56 × 39μ	72 × 36μ	80 × 36μ	60 × 35μ

T. serratus is stated to have a vaginal prolapse while in *T. campanulus* the vulvar lips are slightly salient; the projecting lower vulvar lip in the present species is no doubt the same structure interpreted by von Linstow as a prolapse. The differences between the present species and *T. serratus* are accordingly mainly differences in dimensions, those for body and spicular lengths of the present specimens being roughly one-third of *T. serratus*; the eggs however are almost identical in size. There seems no reason accordingly, why it should not be referred to von Linstow's first species. While the present evidence is too scanty to allow of a definite opinion, it seems probable that only a single species exists in Felidae. It is peculiar however that all records of whipworms in cats should be South American.

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BIOLOGICAL NOTES ON THE CHRYSOPIDAE[1]

By Wm. L. Putman[2]

Abstract

This paper presents further biological and ecological data on the Chrysopidae gathered during a study of the predators of the oriental fruit moth. The species concerned are *Chrysopa rufilabris* Burm.; *C. plorabunda* Fitch; *C. downesi* R. C. Smith; *C. oculata* Say; *C. nigricornis* Burm.; *Meleoma signoretti* Fitch and *M. emuncta* Fitch. Data on life history include relation of development to temperature, number of generations, method of overwintering, and oviposition. The early stages of *C. downesi*, *M. signoretti* and *M. emuncta* are described. Seasonal prevalence and fluctuations in abundance from year to year are discussed and notes are given on natural control agencies. The value of chrysopids in the biological control of the fruit moth is summarized, with the conclusion that only in exceptional seasons are they of appreciable importance.

In a previous paper (1) the writer gave some account of the value of chrysopids as predators of the oriental fruit moth (*Grapholitha molesta* Busck), together with some notes on their habits and life histories. This paper presents further details of studies in which most attention was given to *Chrysopa rufilabris* Burm., *C. plorabunda* Fitch, and *Meleoma signoretti* Fitch, these being the most abundant and the only species of appreciable importance as predators of the fruit moth. More or less incidental notes were also made on *C. oculata* Say, *C. downesi* Smith, *C. nigricornis* Burm., and *M. emuncta* (Fitch). There is some doubt concerning the identity of the species here called *Chrysopa downesi* which is discussed on a later page.

Life History

All the life-history studies were carried on in the screened insectaries at St. Davids and Vineland Station.

Incubation period. It was possible to determine the incubation period of only a small number of eggs as very few were laid in captivity. The length of the period, with the mean daily temperature determined from thermograph records is given in Table I.

TABLE I

Incubation period of Chrysopids

Species	No. of eggs	Length of period, days	Mean daily temperature, °F.
C. rufilabris	26	6 - 7	68.6 - 64.8
C. plorabunda	12	5 - 6	73.3 - 66.6
M. signoretti	5	9	66.1

Larval period. The larvae were reared in glass shell vials plugged with cotton. They were supplied daily with fresh eggs or larvae of the fruit moth

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Contribution from the Entomological Branch, Dominion Department of Agriculture.

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or Mediterranean flour moth (*Ephestia kuehniella* Zell.). The duration of the three larval instars was recorded for a few larvae and appeared to be approximately equal. The length of the complete period in relation to the mean daily temperature is given in Table II.

TABLE II

LARVAL PERIOD OF CHRYSOPIDS

Mean daily temperature, °F.	No. of larvae	Larval period, days	
		Extremes	Average
Chrysopa rufilabris Burm.			
77 - 77.9	3	10	10.0
76 - 76.9	4	9 - 13	10.8
75 - 75.9	26	9 - 12	10.0
74 - 74.9	52	10 - 14	11.5
73 - 73.9	43	9 - 14	11.1
72 - 72.9	35	10 - 15	11.9
71 - 71.9	49	11 - 15	12.6
70 - 70.9	45	11 - 15	12.7
69 - 69.9	18	11 - 15	13.0
68 - 68.9	15	12 - 15	13.3
67 - 67.9	8	12 - 14	12.8
66 - 66.9	1	14	14.0
65 - 65.9	9	14 - 18	15.8
63 - 63.9	3	16 - 18	16.7
62 - 62.9	1	17	17.0
59 - 59.9	2	24	24.0
Chrysopa plorabunda Fitch			
77 - 77.9	1	8	8.0
76 - 76.9	1	9	9.0
74 - 74.9	8	10 - 12	10.5
Chrysopa plorabunda Fitch—*Concluded*			
73 - 73.9	2	10	10.0
71 - 71.9	4	10 - 15	12.5
70 - 70.9	11	10 - 13	11.5
69 - 69.9	10	12 - 13	12.4
68 - 68.9	10	11 - 14	13.2
67 - 67.9	2	12 - 15	13.5
66 - 66.9	1	15	15.0
65 - 65.9	5	16	16.0
64 - 64.9	5	16 - 18	17.0
63 - 63.9	2	17	17.0
61 - 61.9	4	18 - 20	19.5
60 - 60.9	4	19	19.0
59 - 59.9	1	23	23.0
Meleoma signoretti Fitch			
73 - 73.9	1	15	15.0
71 - 71.9	4	15 - 19	16.7
70 - 70.9	3	15 - 18	16.0
69 - 69.9	8	17 - 18	17.6
68 - 68.9	15	17 - 19	18.5
67 - 67.9	2	18 - 19	18.5

Pupal period. The cocoons were left in the vials in which they were spun. There was considerable mortality in the cocoons, ranging from 13 to 37% based on 41 and 187 individuals respectively. Moisture was supplied by wetting the plugs of the vials occasionally. The period as given in Table III includes the prepupal stage, pupation occurring from three to six or more days after the cocoon was spun.

TABLE III

PUPAL PERIOD OF CHRYSOPIDS

Mean daily temperature, °F.	No. of pupae	Pupal period, days	
		Extremes	Average
Chrysopa rufilabris Burm.			
77 - 77.9	3	10 - 11	10.3
76 - 76.9	7	9 - 13	10.9
75 - 75.9	5	10 - 12	11.5
74 - 74.9	13	11 - 13	11.4
73 - 73.9	29	11 - 14	11.7
72 - 72.9	20	11 - 16	13.0
71 - 71.9	27	11 - 15	14.0
70 - 70.9	21	13 - 16	14.4
69 - 69.9	1	15	15.0
68 - 68.9	10	13 - 17	15.6
67 - 67.9	12	16 - 18	16.6
66 - 66.9	2	16 - 17	16.5
65 - 65.9	8	17 - 20	19.0
64 - 64.9	9	18 - 21	19.3
Chrysopa plorabunda Fitch			
74 - 74.9	2	10 - 11	10.5
73 - 73.9	1	10	10.0
71 - 71.9	1	11	11.0
70 - 70.9	6	11 - 14	12.2
69 - 69.9	2	13	13.0
68 - 68.9	2	13 - 14	13.5
67 - 67.9	4	12 - 14	13.3
65 - 65.9	18	14 - 19	18.0
64 - 64.9	1	19	19.0
61 - 61.9	2	20 - 21	20.5
60 - 60.9	1	19	19.0

Oviposition and longevity of adults. It was very difficult to get adults of *C. rufilabris* emerging in the insectary to oviposit, although cages of various types were used and the insects supplied with water and aphids, sugar solution, and yeast. A few eggs were obtained from cages placed on aphid-infested trees but the number normally laid is not known. When supplied with aphids, adults of this species lived as long as 45 days, but they died quickly when given water only.

C. plorabunda was much more easily handled in captivity and usually oviposited when caged on aphid-infested twigs. Adults of the summer generations deposited from 18 to 114 eggs and lived as long as 42 days. Overwintering adults laid more than 56 eggs over a period of five weeks. An examination of the undeveloped ovaries of this species showed a potential production of at least 144 eggs.

Seasonal life history. C. rufilabris overwintered in the prepupal stage within the cocoons. These are spun among the leaves, with which they fall, on the twigs, and in considerable numbers inside the curls of the thin outer bark. The exact dates of spring emergence were not determined but they appeared to be early in June, as eggs were found as early as June 10. There appeared to be two complete generations and probably a partial third, but these were not distinguishable in the field because of the long oviposition period and the great variation in the length of the larval stages under conditions of food scarcity. The number of eggs present in the peach orchards usually increased rapidly from about the middle of June and reached a maximum about the middle of July, after which oviposition decreased rapidly. Eggs were very scarce after early August, although a few could be found till nearly the middle of September. All the eggs found up to and including the time of maximum deposition were laid by the spring brood of adults, and while in some years, particularly in 1933, there was some indication of a slight increase in the egg population in early August, the number laid by the first brood adults was very small and much less than that of the spring brood. The presence of the first brood in considerable numbers was shown by the bait pail catches, so it appeared that the decline in egg population was due to the reduced fecundity of the females of this brood. In the writer's previous paper it was conjectured that lack of food for the larvae was responsible, but an equally probable cause was insufficient nourishment of the adults. The experiments in connection with oviposition outlined above appear to show that aphids are necessary for the proper development of the ovaries. Green peach aphids, *Myzus persicae* Sulz., were usually common in the orchards during the greater part of June and a few usually remained until July, while the spring brood of *rufilabris* was active, but all migrated to other hosts before the appearance of first brood adults.

C. plorabunda hibernated in the imaginal state. Several were successfully wintered on the earth floor of the insectary under an inverted flower-pot. While none were actually discovered in midwinter, they were found in late fall and early spring among dead grass and fallen leaves, in strawberry beds and in outbuildings. On warm days they were often active until late November and were seen in flight as early as March 22. On the approach of cold weather, in late October and November, the usual green color of the species changed to yellow mottled with brown, but reappeared early in May after they emerged from hibernation and began to feed. Both sexes hibernated, the females with undeveloped ovaries, and fertilization took place in the spring. In cages, oviposition commenced about the first of June and continued nearly to July. In the orchards, eggs were not found before June 27 and in some years not until nearly a month later. Apparently the adults did not hibernate in the orchards because of lack of shelter, and did not invade them in noticeable numbers until the time of the late spring brood or early first brood oviposition. After this time eggs could be found during most of the summer, sometimes till September 11, but there were no consistent periods of maximum oviposition and the broods could not be distinguished. As this species produces eggs earlier in the season and the developmental period is somewhat shorter, three complete generations are possible, but it is doubtful whether the third is very large.

Meleoma signoretti passed the winter as a prepupa within the cocoon. Adults emerged between June 2 and 5 from a few cocoons overwintered in the insectary. In 1932, the only year in which any appreciable numbers were present, the first eggs were found in the orchards on July 5, the maximum number on July 20 and the last on August 24. There was one complete generation and, at least in some years, a partial second. In 1931, first brood adults were taken in the bait pails from August 18 to September 8, while in 1932, when the species was much more abundant, none were taken, and larvae maturing as early as August 7 did not emerge as adults that season.

Food Habits of the Larvae

Larvae of *C. rufilabris* were seen in the orchards attacking eggs and larvae of the oriental fruit moth, nymphs of the cottony peach scale (*Pulvinaria amygdali* Ckll.), European fruit lecanium (*Eulecanium corni* Bouché), white fly (*Trialeurodes vaporariorum* Westw.), the leaf-hopper, *Erythroneura obliqua* Say, and all stages of the European red mite (*Paratetranychus pilosus* C. & F.). They would apparently destroy almost any soft-bodied insect not too large or active to handle, but could not be reared satisfactorily on green apple aphids (*Aphis pomi* DeGeer) and also refused the woolly species of aphids.

There was less opportunity for observing the food preferences of *C. plorabunda* and *M. signoretti*, but these species and *C. downesi* ate fruit-moth and flour-moth eggs and larvae and aphids in the insectary and were apparently similar to *rufilabris* in their choice. *C. oculata* appeared to be chiefly an aphid feeder, as only a few larvae could be induced to accept fruit-moth eggs and then only when starved. The habits of *C. nigricornis* appeared to be very similar.

Fluctuations in Abundance

As part of an ecological study of the oriental fruit moth, population studies of chrysopid eggs were made from 1931 to 1935 at four selected orchards in the Niagara Peninsula. These were located at St. Davids, Niagara-on-the-Lake, Vineland Station, and Grimsby Beach, covering about 22 miles of the fruit belt. Briefly, the procedure was to make one-hour counts at chosen points in the mature orchard at weekly intervals from approximately June 1 to September 10. Duplicate counts were also made on young trees at each point, but because of the variation in the age of the trees and the necessity of choosing a new block as the original one became too old, it was thought that the figures were not comparable and were discarded. Approximately a thousand leaves were carefully examined at each hourly count and all fruit-moth eggs and other insects also recorded. The chrysopid eggs were collected and reared to determine the species. In Table IV the combined counts for each season are given.

TABLE IV

CHRYSOPID EGG POPULATION IN PEACH ORCHARDS

	1931	1932	1933	1934	1935
C. rufilabris	508	11	83	262	119
C. plorabunda	90	19	5	16	19
C. nigricornis	—	32*	—	—	—
C. oculata	—	6	18	68	19
M. signoretti	—	34	3	—	1

* *1 cluster of 32 eggs.*

Unfortunately no counts were made in 1930 when chrysopids were more abundant than in any succeeding year. A perusal of the table will show a great variation in both the abundance of each species during the five-year period and the relative numbers of the species in each year. *C. rufilabris* was by far the most prevalent species in every year except 1932, when it was relatively scarce; it occurred in greatest abundance in 1931 and was again very common in 1934. *C. plorabunda* was always much less abundant except during 1932, and was also most prevalent in 1931. *M. signoretti*, usually an uncommon species, suddenly became quite common in 1932, but very few were found during succeeding years. The few eggs of *C. oculata* were collected largely during June, while aphids were still present on the trees, and it should be noted that the greatest numbers were found in 1934, a year of unusually heavy aphid infestation. Eggs of *C. nigricornis* were found on only one occasion.

The larvae of *rufilabris*, *plorabunda* and *signoretti* were commonly observed on the trees in numbers roughly proportionate to the egg populations. Exceedingly few *oculata* were found, the larvae apparently migrating to the soil or low vegetation. One or two larvae of *C. downesi* were collected and a few eggs found in another orchard.

Natural Control Factors

Infertility of eggs. Approximately 5 to 9% of the eggs collected in the orchards failed to hatch. These figures were derived from collections of 103 and 180 eggs respectively. Some of this mortality might have been due to injury in collecting, but many eggs appeared to be infertile.

Egg parasitism. Parasitism of the eggs by *Trichogramma minutum* Riley ranged from traces in 1933 and 1934 to 5% of counts of 290 and 179 eggs in 1932 and 1935 respectively, and 7% of 987 eggs in 1931. In individual orchards it reached 12% of 221 eggs examined in 1931 and 50 examined in 1932. Chrysopid eggs in years of abundance may be of some importance as a reservoir in which a stock of *Trichogramma* is built up to attack the later generations of the fruit moth.

Food supply of larvae. Lack of sufficient food appeared to be one of the most important factors limiting the chrysopid population. In some years chrysopid eggs were often the most prevalent form of life on the trees and under such conditions very few larvae could survive.

Pupal parasites. These are not properly pupal parasites as they attack the prepupa. The only primary parasite discovered was *Chrysopoctonus rileyi* Ashm. which was reared several times from summer generation cocoons of *rufilabris*, *plorabunda*, and *nigricornis* and probably attacks all species. The most abundant species was *Hemiteles tenellus* Say, reared many times from cocoons of both summer and overwintering generations of *rufilabris* and *plorabunda*. An examination of the contents of the cocoons proved that this species was hyperparasitic, probably on *Chrysopoctonus*, and in view of its abundance must be a decided check on parasitism by the latter species. One specimen of another hyperparasite, *Perilampus chrysopae* Cwfd., was obtained, and a few undetermined chalcids emerged from another cocoon. It was impossible to determine the extent of parasitism at any one time because of the scarcity of cocoons, but a lot of 31 old cocoons collected in 1932 from peach trunks, and representing all generations for two years or more showed a parasitism of approximately 50%.

Destruction of cocoons by predators. Some of the cocoons found on the trunks had been torn open by some predator and the pupae devoured. Some coleopterous larvae resembling *Tenebroides* were found in the same location and may have been responsible.

Natural mortality within the cocoon. Many cocoons were found containing dead pupae or prepupae. Among those collected on the trunks this natural mortality reached approximately 26%.

Food supply of adults. The possibility that a deficient food supply may limit the fecundity of the females has been discussed on a previous page.

Summary. The most important factors limiting the chrysopid population are lack of food for the larvae and possibly for the adults, and parasitism; but the manner in which these and probably other unknown influences, such as weather conditions, cause the great fluctuations in numbers is not known.

Descriptions of Immature Stages

The early stages of most of the species concerned have been well described and figured by Smith (2) and only those not previously known are described here.

Chrysopa downesi Smith

There may be some doubt concerning the identity of the species here called *downesi*. A small series was identified as this species by its author, Dr. R. C. Smith, but none of the hundred or more examined by the present writer have the black band over the genae mentioned in the original description (3), but have a red band only, in this respect agreeing with *harrisii* Fitch. There is some variation in size and the relative widths of the wings but only one species appears to be represented. The adults in life have a characteristic bright, deep green color which soon disappears in mounted specimens.

Egg. Stalked; yellowish-green; indistinguishable from that of *plorabunda*.

Larva. Unfortunately no detailed description of this species was made, but the only noticeable distinctions between it and that of *plorabunda* were the markings of the head. These consisted of two longitudinal bands converging somewhat toward the base of the head, as in *plorabunda*, but of uniform width throughout and not expanding at the base as in the latter species.

Cocoon. Oblong-spherical, of pale yellow-green silk. Cocoons of other species may appear greenish but this is due to the enclosed pupa. Length 3.2 mm.; width 2.8 mm.

Meleoma signoretti Fitch

Egg. Of typical chrysopid type; stalked; deposited singly.

First instar larva. General color pale. Head with two conspicuous longitudinal, somewhat reniform black marks, concave toward the middle line, very similar to the second instar. Smith's figure ((2), Plate LXXIV) doubtfully referred to this species, is apparently correct. Thoracic segments each with two large dark spots. Abdomen with obscure darker markings. Tubercles with long, curved pale setae. Legs pale, apices of segments ringed with dusky. Width of head, 0.39 mm.; length of mandibles, 0.39 mm.; antennae, 0.57 mm.; longest setae, 0.52 mm.; total length, 2.07 mm.

Second instar larva. (Fig. 1, a). Head similar to first instar but spots more angular; jaws pale amber, darker apically; antennae and palpi pale brown. Thorax pale yellowish; prothorax with two dark longitudinal im-

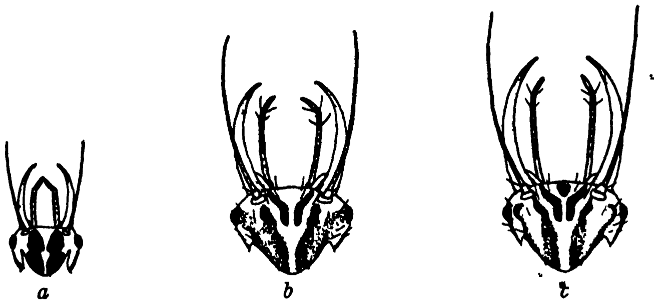

Fig. 1. *a. Meleoma signoretti; second instar larva. b. M. signoretti; third instar larva. c. M. emuncta; third instar larva.*

pressions (sclerites) and marked with pale reddish; meso- and metathorax each with two brown marks on either side, the posterior larger and extending diagonally outward and backward to small black impressions; pale reddish spots external to brown ones; dorsal vessel brown with lighter brown irregular stripes on either side; tubercles pale marked with reddish. First abdominal segment without tubercles; those of second segment marked with brown. Abdomen pale yellow, with irregular broken brown markings tending to form two bands laterally which become darker and converge toward the caudal extremity; brown markings interspersed with pale reddish. Thoracic and abdominal tubercles well developed, with curved pale setae. Legs pale. Width of head, 0.74 mm.; length of mandibles, 0.59 mm.; antennae, 0.98 mm.; longest setae, 0.50 mm.; total length, about 5 mm.

Third instar larva. (Fig. 1, b.) Head with two pairs of curved black marks, the inner on the anterior part of the head, extending forward, diverging and angled outward to the base of the jaws; outer pair shorter and broader, not extending as far anteriorly and merging into broad pale brown bands extending posteriorly to the base of the head; from the outer side of these, similar broad bands extend diagonally outward and forward to the eyes; a dark band extending along side of head from base nearly to eyes; eyes black. Jaws amber brown, darker apically. Antennae and palpi pale brown. Thorax and abdomen similar to second instar but markings paler and more diffuse; black impressions on prothorax divided longitudinally and a central black spot above dorsal vessel. Width of head, 1.04 mm.; length of mandibles, 0.95 mm.; antennae, 1.45 mm.; longest setae, 0.75 mm.; total length, approximately 10 mm.

Cocoon. Somewhat oblong spherical; white; loose outer layer sometimes with a reddish tint when new; length 3.8 mm.; width 3.3 mm.

Meleoma emuncta (FITCH)

Egg. Indistinguishable from that of *M. signoretti;* laid singly.

First instar larva. Head with two large longitudinal black marks which meet along the middle line, leaving triangular light areas at base and apex of head; a narrow black line in the light areas on either side of the large marks extending from the base half the length of the head; regions about the eyes black; mandibles amber-colored. Thorax and abdomen pale reddish brown; lateral tubercles white except second abdominal which are dark; setae long and curved, pale. Legs pale.

Second instar larva. Head similar to first instar; ground color pale yellowish gray. Thorax pale yellowish; prothorax marked with pale reddish and with two longitudinal black impressions; mesothorax and metathorax marked with brown, tending to form two oblique bands converging posteriorly on each segment with a smaller spot external to them; sides of mesothorax below and behind lateral tubercles dark; tubercles pale, marked with pale reddish, large and prominent. Abdomen darker yellowish, finely marked with brown, more heavily and tending to form two bands toward the caudal extremity;

dorsal vessel pale grayish brown, bounded by paler lateral areas; sides of second abdominal segment, including lateral tubercles, dark brown to black; tubercles on other segments finely spotted with brown on bases; setae on all tubercles long, curved, partly black.

Third instar larva. (Fig. 1, c.) Head pale yellowish marked with dark brown or black; from centre of head two black bars extend parallel anteriorly and then diverge sharply toward bases of jaws; a median triangular black spot between the bands near the anterior margin; two short oblique bars external to central bands; from these bars paler brown bands extend forward to bases of antennae and backward to base of head where they become much broader and give off similar bands obliquely outward nearly to eyes, enlarged, darker, and frequently somewhat crescent-shaped at the apices. Mandibles amber-colored, darker apically. Antennae and palpi pale brown. Thorax and abdomen similar to second instar, but brown markings paler and interspersed with pale reddish; sides of mesothorax pale. Legs pale. Width of head, 1.14 mm.; length of mandibles, 1.00 mm.; antennae, 1.54 mm.; total length, about 10 mm.

Cocoon. Similar to that of *M. signoretti.* Length, 4.00 mm.; width, 3.3 mm.

The larvae of *Meleoma* are easily distinguished from those of *Chrysopa* by their yellow-brown color and by their head markings. *Emuncta* larvae can be separated from *signoretti* by the median triangular spot near the apex of the head in the former species.

Influence of Chrysopids on the Oriental Fruit Moth

The only species with such habits and occurring in such numbers that they are potential factors of importance in the control of the fruit moth are *Chrysopa rufilabris*, *C. plorabunda*, and *Meleoma signoretti.* There is little to add to the writer's account of their destruction of eggs given in his previous paper (1), but they have since been found occasionally attacking larvae in the field. Unfortunately we still have no method of giving a numerical value to their influence, but there is no doubt that in 1931, and still more so in 1930, chrysopids were of considerable importance in the control of the moth, but from 1932 to 1935 they were apparently of minor value. The period of greatest abundance coincides very closely with the time the second brood eggs are laid and the greatest check is exerted on this brood.

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STUDIES ON THE HETEROPHYID TREMATODE *APOPHALLUS VENUSTUS* (RANSOM, 1920) IN CANADA

PART II. LIFE HISTORY AND BIONOMICS[1]

By Thomas W. M. Cameron[2]

Abstract

The eggs of the trematode pass into water, embryonate and are swallowed by the snail, *Goniobasis livescens*. They do not hatch in water. In the snail, multiplication through redia and daughter redia stages takes place and cercariae, with long flanged tails and pigmented eyespots, are produced. These escape into the water, but have a free life of less than 48 hours. To survive during this period, they must penetrate the skin of a fish, a great variety of which are successful intermediaries. In the muscle of the fish, the cercaria encysts to become metacercaria, which is however not immediately infective. Infection of the definitive host is by ingestion of the uncooked fish.

When *Apophallus venustus* was found to be endemic in the lower Ottawa Valley, preparations were made to attempt to elucidate the life cycle. While the life history of all the species in this genus was quite unknown, it was known that, in such other members of the family as had been studied, two successive intermediaries were essential. The second of these was always a fish, and the first an operculate snail.

Accordingly, a large number of freshwater fish was obtained locally and fed to kittens which had been raised in the college and had never been fed on fish. With a very few exceptions, every species obtained was found to be infective. *Apophallus venustus* was obviously, like the other members of the family, carried by fish.

The search for the snail intermediary was more difficult. By analogy from what was known of the other members of this family, it should prove to be an operculate snail. Moreover, all the cercariae already known from the Heterophyidae have pigmented eye-spots and peculiar flanges on their tails. Finally, the high incidence of infection in the fish suggested that the snail vector would be common locally and that a large percentage would be infected.

Search was accordingly made in the first instance for common species of operculate snails in the Ottawa River. Five species were found and I wish to acknowledge the invaluable assistance of Mr. A. LaRocque, of the National Museum, Ottawa, in identifying these.

The commonest local operculate snail was *Amnicola limosporata* which was found in thousands on the stems of various water weeds. Several hundreds of these snails were crushed and examined microscopically. No trace of any kind of trematode larva was found.

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[1] *Manuscript received November 3, 1936.*
Contribution from the Institute of Parasitology, McGill University, Macdonald College, Quebec, with financial assistance from the National Research Council of Canada.

[2] *Research Professor of Parasitology, McGill University; and Director, Institute of Parasitology, Macdonald College, P.Q., Canada.*

Campeloma decisum, a large species living on decaying vegetable matter, was also collected in some numbers, although it was much less common. No cercariae were found in this snail either.

Bulimus (Bithynia) tentaculatus is also very common locally, although it is a species introduced from Europe. This made it an improbable intermediate host for an endemic American trematode; nevertheless large numbers were examined but all proved negative.

These three species were subjected to artificial infection without result.

A species of *Pleurocerca*, a St. Lawrence form occurring in the Ottawa River only at its point of junction with the St. Lawrence River at Lake St. Louis, was obtained in small numbers. None was infected.

The remaining species, *Goniobasis livescens*, was first collected from stones just off Lynch Island, two miles east of Ste. Anne de Bellevue. It was present in large numbers and an examination of the first batch of 82 snails showed that 31% were infected with a cercaria which was very similar to that described by other workers from various species of Heterophyid trematodes. It seemed probable that this was the larval stage of *Apophallus venustus*. No other species of Heterophyid has been found locally in wild or domestic mammals or birds, and no other heterophyid cercaria has been found in any other species of local snail, although large numbers of all the common species, both operculate and non-operculate, have been carefully examined by my colleagues and myself in the laboratory during the past three years in connection with this and other trematode life cycles.

Positive proof of the life history of a trematode depends upon the production of all stages in the laboratory, in animals raised under circumstances which absolutely preclude any accidental contamination. In this particular case, it postulates laboratory-bred cats, laboratory-bred fish and laboratory-bred snails. The first of these conditions was easily fulfilled and the cats used have definitely not been exposed to natural infection. Attempts to breed suitable fish (such as catfish and black bass) in the laboratory under conditions which would satisfy these postulates, were unsuccessful and this was likewise the case with *Goniobasis livescens*.

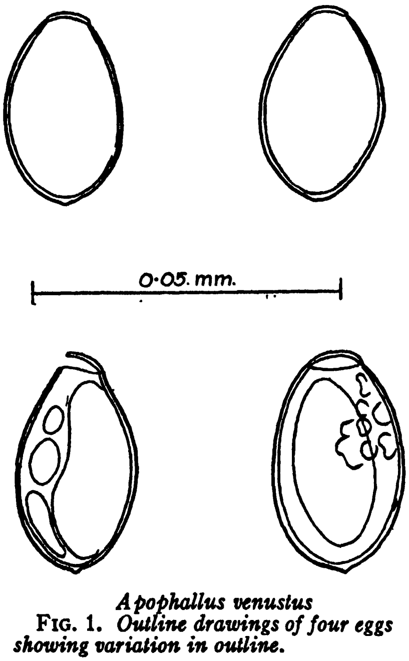
Early in 1935, a large number of very young catfish had been collected and kept in the aquarium at the Institute of Parasitology. A number of these died from one cause or another but no encysted cercaria could be found on compressing the whole fish in a trichina compressorium. Others were later subjected to infection with the cercariae emerging from the snails and the larval flukes observed actively penetrating the skin of the fish. Experimentally infected fish were killed and examined from time to time and the encysted cercariae in the flesh were observed to develop and require some time to become mature. Negative results followed the feeding of cats with catfish containing young cysts and it seems reasonably sure that no previous infection with this fluke was present in the fish. One of these fish, a month after its last exposure to infection, was fed to a kitten; three weeks later the cat was autopsied and *Apophallus venustus* found in its intestine. These experiments did not, however, furnish absolutely conclusive proof of identity.

A number of catfish was collected from an artificial pond on the grounds of Mr. J. J. Harpell, of the Garden City Press, Gardenvale, Quebec. These were young fish, bred in this pond which contained no operculate snails of any kind. They were retained in the laboratory for a month after experimental infection, and then fed to a cat. The result was positive. At the same time, a number of catfish from the same pond was collected and fed directly to another cat, without being exposed to the cercariae; the control was negative.

Thus the entire life cycle has not been observed in an entire experimental series. However, the high percentage of infected *Goniobasis livescens*, the presence of a single species of heterophyid cercaria in the area, and of a single species of adult heterophyid trematode in all the local animals examined, together with the artificial infection of fish and the large number of negative controls, is quite conclusive evidence that all the stages found belong to the same species of trematode.

Egg-miracidium Stages

The egg is more or less oval in contour but varies quite considerably in different specimens (Fig. 1), In a few it is distinctly constricted at the opercular end and the operculum is quite obvious; in others, it is an almost perfect oval and the operculum is seen with difficulty. Frequently there is a small thickening at the end opposite the operculum. In size it varies from 26μ to 32μ long and 18μ to 22μ wide, the mean size of a considerable number being 29μ by 20μ. In color it is light brown, and the outer surface has many honey-combed, irregular lines on it. When deposited in the intestine, it is not embryonated and I am unable to state the exact time necessary for the development of the miracidium. In the laboratory, in shallow water in Petri dishes, at least two weeks were required but further data on this are necessary.



Apophallus venustus
FIG. 1. *Outline drawings of four eggs showing variation in outline.*

The egg does not appear to hatch naturally, and specimens kept in the laboratory for almost a year did not hatch. As the pressure necessary to force off the operculum also invariably destroyed the miracidium, I have been unable to ascertain details of this larval stage. It does not however, completely fill the egg shell, which also contains several large globules of a fat-like substance (Plate I, Fig. 2). Its general appearance is very similar to that of *Cryptocotyle* and *Opisthorchis*. The miracidium appears to hatch only after ingestion by the snail. Several snails, from a stock known to be parasite-free, were exposed to infection by placing them for a few hours in a Petri dish, the bottom of which was covered with washed feces containing eggs. The snails were then removed and placed in a clean aquarium jar and ultimately mother-redia were found in them. The snails unfortunately died at this stage, probably as the result of superinfection.

The culture of eggs was examined immediately after the removal of the snails and no hatched eggs were seen. This removes the possibility of some special stimulus, associated with the snail, being responsible for hatching, and leaves actual ingestion as the only alternative.

Snail Host (Plate I, Figs. 3, 4, 5)

As mentioned in the introduction, the only species of local snail found naturally infected was *Goniobasis livescens*. They are relatively abundant in parts of the Ottawa and St. Lawrence Rivers, near Ste. Anne de Bellevue, in areas where the necessary environmental conditions are found.

The genus has its centre of distribution in the southern United States, and LaRocque* is of the opinion that only two species occur in eastern Canada, *viz.*, *G. haldemani* in Lakes Erie and Huron and *G. livescens* in the lakes and rivers of the drainage area of the Great Lakes and St. Lawrence—except those emptying into the St. Lawrence below the salt water line. All the specimens of *Goniobasis* used in the present work appear to belong to this species.

Goniobasis livescens has a thick, solid, more or less spiral, elongated shell, with a subrhomboidal aperture, closed by a subspiral horny operculum. There is a tendency among conchologists to divide this species into varieties which correspond with differing habitats. Its general habitat is a boulder bottom or on stones with sandy spots. Its presence in the latter area can usually be traced by the deep furrows which it makes as it moves along the sand. It is generally found in from one to three feet of water, in situations exposed to the river current, where it feeds on desmids and diatoms.

Baker (1) describes its behavior in an aquarium as follows:

"It will glide up the side of the glass jar with as much celerity as a *Lymnaea* or *Physa*, though not as rapidly. When at rest on the bottom, it moves more or less slowly, pushing its foot forward a short distance and drawing the shell after it, a sort of stepping process. While in motion, the rostrum is constantly in motion searching for food, the action of the radula being plainly seen as it scrapes the stones and other objects. The mouth may be opened and the radula seems to be used in a lapping manner. The long tentacles are constantly in motion, being used as tactile organs feeling and testing all objects within reach. Sight is apparently deficient, the tentacles performing the function of this sense."

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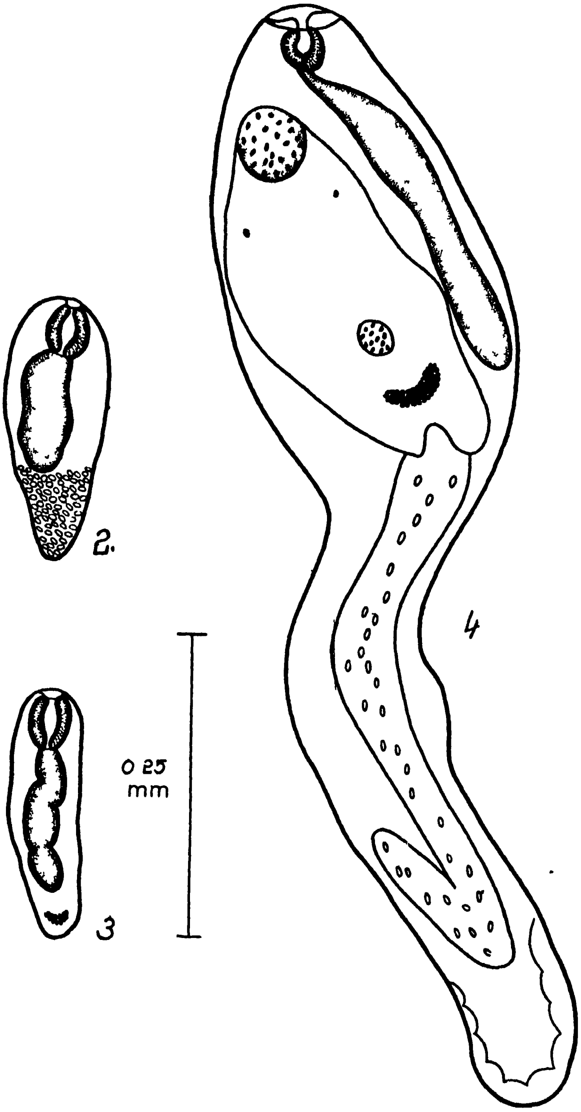
Our experiences with this species are fully in accord with Baker's.

While *Goniobasis* occurs in a variety of places in the local waters, the most plentiful sources of supplies were found to be the Isles de la Paix on the St. Lawrence and the east end of Lynch Island, about two miles down the Ottawa River from Ste. Anne de Bellevue. Large numbers were collected from both these areas. All snails were examined for infection shortly after being brought into the laboratorv and at intervals during the succeeding two months. The specimens that died were examined by crushing and searching under the binocular dissecting microscope. Living snails were placed individually in quarter-pint glass cream bottles, filled with water and examined with a hand lens for escaped cercariae 24, 48 and 72 hours later. Negative examples were placed in a stock jar, positive examples used as a supply of larvae. Negative examples were re-examined periodically and further segregated. In no case was any naturally infected snail found in the St. Lawrence islands. However, a large number of infected snails was found near Lynch Island in the Ottawa River; in one batch of 82 examples (part of a single day's collection) 26 infected snails were found—an infection rate of 31.6%. As such natural bird hosts as might exist or have been discovered are common to both areas, I can give no reason for this heavy infection rate or for the complete absence of any infection on a group of islands in the St. Lawrence, only four miles distant. In the spring of 1936, a large number of snails of this species was obtained from the Rideau River in Ottawa city. Two hundred of these were crushed and examined; two contained the cercariae of this trematode and two contained rediae and daughter rediae which may also belong to the same species, but which were too immature to identify.

Intra-molluscan Development

There has been little opportunity to examine the intra-molluscan development of the parasite and, as mentioned above, experimental infection was carried only as far as the mother-redia stage. The sporocyst stage has not been identified and all subsequent stages occur in the liver (Plate I, Fig. 6).

Young mother rediae were seen only in experimental infections, infected on October 30, 1935, and examined on November 16. At that stage of development, they are carrot-shaped organisms, about 0.22 mm. long (Fig. 2), with an almost spherical oral sucker at the blunt end, and a very large single caecum extending posteriorly for about two-thirds of the body length. The remainder of the body is filled with spherical cells. These rediae become much larger and are ultimately filled with daughter rediae (Fig. 3), which, in their young stages, are small oval bodies with an oral sucker and a single caecum. The daughter rediae can move about by vermiform contractions of the body, but possess no hooks or spines. When fully mature (Fig. 4), they are elongated sausage-shaped bodies, with no appendages and apparently no functional birth pores. They contain a varying number of cercariae—from one to eight—rarely more (Plate I, Figs. 7, 8). These cercariae are fully developed morphologically before they leave the daughter rediae.



Apophallus venustus

Figs. 2, 3 and 4. Fig. 2. *Young mother redia.* Fig. 3. *Young daughter redia.* Fig. 4. *Mature daughter redia, containing a single cercaria. All drawn to same scale.*

Cercaria

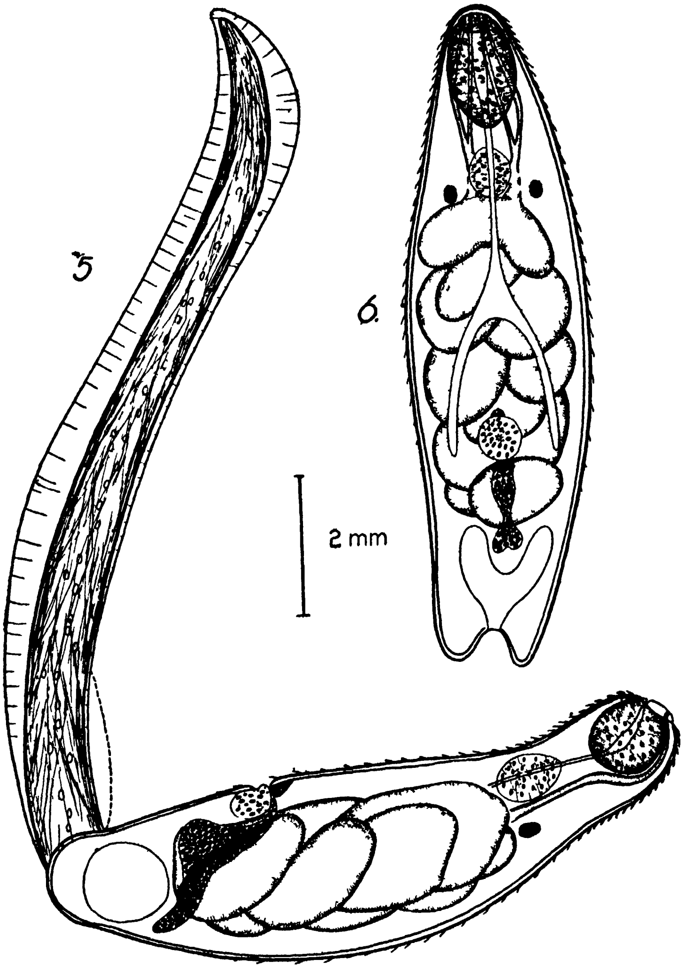
The cercaria (Figs. 5, 6) is a very muscular larva and subject to great changes in body shape. This description is based on relaxed, living specimens, supplemented by sections and whole mounts of cercariae killed in formalin and stained with alum carmine. It has a long tail about one and one-half times the body length—sometimes a little longer, but never more than twice the body length. It is provided with a complete dorsal and an incomplete ventral fin of a very delicate structure (Plate II, Fig. 1). The dorsal fin originates as a very small fold close to the body and, gradually increasing in width, reaches its maximum about half-way down the tail; thereafter it becomes slowly reduced in size. It is continued around the ventral side to expand again to form a ventral fin, which however terminates about the mid-point of the tail. In a minority of specimens, there is a second ventral fin close to the body (shown as a dotted line in Fig. 5). The membranous fin is longer at its free edge than on its attached edge and it is, in consequence, undulated. This tail is very muscular and is attached, by a "ball and socket" mechanism to the posterior end of the body. It is very easily detached by its own rapid movement.

The body is about 0.10 to 0.13 mm. long and about one-third of this in width (Fig. 6). The oral sucker is spherical to oval in shape and only feebly muscular. It is about 20μ in diameter. It is sub-terminal in position and the mouth opening is towards the ventral surface. There are no hairs on the body. However, the whole surface of its anterior part to about its mid-point, is thickly covered with minute spines (as in the adult) except for a small area surrounding the termination of the oral sucker and just dorsal to it. The spines at the edge of this area are slightly larger than the rest. The spines on the posterior half of the body are few in number. The oral sucker is pierced by the oesophagus, which continues as a minute simple tube, to a point just in front of the middle of the body. There the tube bifurcates, and in one specimen the bifurcations were traced as far as the level of the ventral sucker. About mid-way between the bifurcation and the anterior end, the oesophagus is surrounded by an oval muscular pharynx.

Dorsal to the pharynx are two darkly pigmented eyespots of varying size.

The ventral sucker is rudimentary and appears to have no muscular fibres at this stage. It is however, provided with a small central depression. Just anterior to this is another small depression, surrounded by a mass of small cells—the rudiments of the genital sinus complex (Plate II, Fig. 4).

Passing from this point, dorsally and posteriorly, is a mass of cells, which stains deeply in preserved specimens and obviously represents the genital primordia. This mass (Plate II, Fig. 2) expands at about its mid-point and then commences to form two terminal swellings. Viewed from the side (Fig. 5) it is roughly "L" shaped. On the lateral margins of the body posterior to this are pigmented areas, and between these two areas lies the "Y" shaped excretory vesicle. I was unable to trace the excretory system in detail, although a number of flame cells was observed in different parts of the body. Practically the whole of the centre of the body is filled with 16 large glandular cells which are readily demonstrated by vital staining. The ducts of these cells pass anteriorly in two medial bundles, which divide into four bundles of four ducts each, just before the oral sucker is reached. These pass forward in a dorso-lateral direction, to open on the spineless area just dorsal to the oral sucker.



Apophallus venustus

FIGS. 5 AND 6. FIG. 5. *Cercaria, side view.* FIG. 6. *Body of cercaria, from ventral aspect.*

This spineless area can be protruded slightly and this enables these glands to come into close contact with the skin of the fish and assist in penetration. Although there is no proof of the fact, it is probable that these glands secrete a histolytic ferment used in penetration. It is possible that a negative pressure from the oral sucker helps to maintain the larva in place during the time this digestive process takes place; the ventral sucker appears to play no part in the action, at least in such specimens as were observed in the act under the microscope.

This cercaria, while possessing the same general characters as other members of this family, differs from them in detail. From the cercaria of *Cryptocotyle lingua* (which is the nearest relation of this species, the life cycle of which is known, and which develops in the marine gastropod, *Littorina littorea*), it differs in its shorter tail, smaller oral sucker, 16 instead of 18 glands, and in the presence of a rudimentary ventral sucker. (5)

The conditions of emergence of the cercariae are not yet fully understood. Temperatures within the limits prevailing naturally (18° C. to 24° C.) appear to have no effect. There was, however, a definite time influence which may be connected with light intensity. From a number of snails examined at hourly intervals during the day (10.00 a.m. to 12.00 midnight), it was found that none emerged before 4.00 p.m., a few between 5.00 and 8.00 p.m. and most between 8.00 and 10.00 p.m. Few emerged during the period between midnight and 10.00 a.m., and the optimum time appeared to be 8.00 to 9.00 p.m. These experiments were conducted at the beginning of October. All cercariae died within 48 hours of emergence in aquaria in the laboratory.

In jars in the aquarium, there is a definite tendency for the cercariae to congregate on the side next to the windows and towards the top of the water. They are most strongly attracted by direct light (on a southern window sill) and when the sun is obscured by a cloud they spread out and become less concentrated and less active. Artificial shadows have a similar effect. They are not only positively heliotropic, but are stimulated by light. This causes a reduction in their free life, and cercariae exposed in a north window do not live more than 24 hours, while those kept in complete darkness may survive for 48 hours.

They swim head foremost, with rapid figure-of-eight lateral lashings of the tail; this is done so rapidly that the entire larva becomes a blur. At the end of this rapid movement, the cercariae become suddenly quiescent and lie in the water with the body horizontal, the dorsal surface being downwards and the tail upwards at right angles to the body. In this position, they slowly sink in the water, suddenly to start rapidly swimming again. The swimming is generally vertically upwards and is generally carried out in short spurts of a few seconds each, the cercariae resting and falling a short distance between spurts. Only occasionally do they move in a diagonal direction.

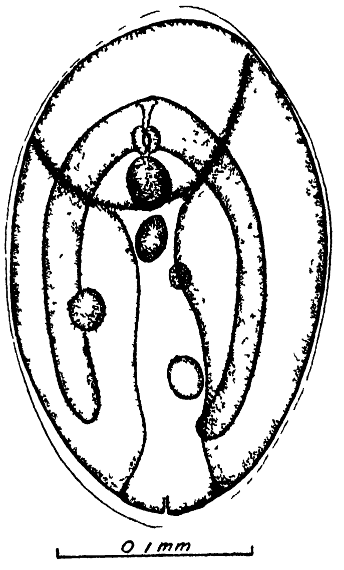
In addition to moving through fluid by the action of their tails, the cercarial body is very muscular and it is able to crawl actively—especially after losing its tail—by worm-like contractions and expansions of the body.

Contrary to Stunkard's experience with *Cryptocotyle lingua*, penetration of the fins by the cercaria can be observed with ease. Young catfish, about two inches long, are easily confined in a Petri dish of water which is placed on the stage of the binocular dissecting microscope. The cercariae, collected after emergence from the snail, swim about rapidly in the water for a short time. As soon, however, as one comes into contact with a fin—the body of the fish appears to have little attraction for them—it holds itself against the skin by violent movements of its tail. These movements are so violent, that the tail is quickly detached. Meanwhile, the cercaria has been evidently digesting its way into the skin by means of the contents of its glands. It generally selects a position near the free edge of the fin, where the skin is thinnest, and within ten minutes it has entered. It generally succeeds in entering a blood vessel up which it moves by alternate contraction and expansion of its body, and it may be assumed that its spines are of considerable assistance in the process. Within 15 minutes of exposure to infection, the tail-less cercaria is seen disappearing into the body of the catfish and small drops of blood may be seen escaping from the open wound at the tip. This process has been repeatedly observed in these small fish. The young catfish are unable to stand the shock of too many penetrating cercariae and a number died from their effects. The bony dorsal and anal fins are the sites of election, although, less frequently, the tail fin also is attacked. Cercariae have never been seen attacking the fleshy dorsal fin of these fish, nor have metacercariae been found in the actual skin of any species of fish; they are always under it or in the muscles. The cercaria encysts in the musculature to become the metacercaria and it is further surrounded by an adventitious cyst formed by the fish itself.

The Metacercaria

The metacercaria (Plate II, Figs. 5, 6), after encystment in the fish, requires considerable time to become mature and infective. Experimental evidence is still lacking as to the exact time necessary, but it appears to be about four weeks. Once mature, probably it can live for a very long period—certainly over winter.

The mature metacercaria is virtually a young adult enclosed in a thin-walled, strong, elastic, hyaline cyst, which in turn is surrounded by a thick-walled fibrous cyst. This cyst is generally oval but varies considerably in size; the largest seen was 0 25 mm. long by 0 175 mm. wide. In this, folded on its ventral face (Fig. 7), lies the metacercaria, and in it the digestive system, ventral sucker, and genital rudiments can be observed. In addition, there is a mass of dark pigment in the excretory vesicle. In the young cyst, remnants of the penetration gland cells and ducts are still visible; these however gradually disappear. When freed from this cyst artificially, a young fluke (Plate III, Figs. 2, 3), about a half-millimetre long, is observed, in which the complete digestive tract, both testes, ovary and a large excretory vesicle filled with this black pigment can be seen.



Apophallus venustus

FIG. 7. *Metacercaria, still enclosed in cyst, but with fibrous outer cyst removed by digestion.*

Fish Hosts

No attempt was made to examine these fish for the presence of metacercariae (other than experimentally infected catfish and the catfish described later) and reliance was placed on experimental feeding of cats. These cats were all raised locally and had not previously been fed on fish. They were placed in cages and each one fed on a single species of raw fish, collected from the Ottawa River.

During 1935 and 1936 the following fish (4) were fed to individual cats:

Scientific name	Popular name	1935 experiments	1936 experiments
Lepisosteidae			
Lepisosteus osseus	Garpike	Positive	—
Catostomidae			
Catostomus commersonii	Sucker, common	Positive	Negative
Moxostoma aureolum	Sucker, red-horse	- -	Negative
Cyprinidae			
Luxilus cornutus	Dace	Positive	- -
Cyprinus carpio	Carp	Positive	—
Ameiuridae			
Ameiurus nebulosus	Bullhead or catfish	Positive	Positive
Esocidae			
Esox lucius	Northern pike	Positive	Positive
Percidae			
Stizostedion vitreum	Wall-eyed pike (or Doré)	Negative	Positive
Perca flavescens	Perch	Positive	Negative
Centrarchidae			
Micropterus dolomieu	Small-mouth black bass	Positive	Positive
Eupomotis gibbosus	Common sunfish	Positive	Positive
Amblophites rupestris	Rock bass	Negative	Negative

Only seven red-horse suckers were available for feeding purposes in 1936, and so no conclusions can be drawn from the negative results. However, 323 perch were fed with no result in 1936 and the infection from these fish in the previous season was a light one. Forty-nine wall-eyed pike in 1936 gave also a very light infection and it seems reasonable to conclude that these are both "poor" hosts. The number of rock bass fed in 1935 was not recorded but it was large, while in 1936, 43 were fed; in no case did an infection result. This result is peculiar as the closely related small-mouth black bass is an excellent host and the sunfish a good one. The best host however was the common bullhead or catfish, and one cat fed on thirty of these fish yielded 5,882 flukes on post-mortem examination.

Ciurea(3), who has published a very detailed account of the metacercaria of the European *Apophallus donicus* as it occurs naturally in fish in Rumania, finds that it occurs in *Perca fluviatilis*, *Lucioperca lucioperca* and *L. volgensis* as well as in *L. sandra*, *Percarina demidoffi*, *Acerina cernua*, *A. schraetster*, *Aspro streber* (among the Percidae) and in *Scardinius erythrophtalmus*, *Abramis brama* and *Blicca björkna* (among the Cyprinidae). In light infections, the metacercariae are found exclusively in the fins, while in heavy infections they are also found in the skin or under the scales. In the fins, they are generally in the interior rays and rarely in the membranes; the caudal and pectoral fins are mainly infected. In the skin, they are generally in the deep layers and only in massive infections are they superficial.

The distribution of the cysts of *Apophallus venustus* has only been studied in the catfish. A number of these fish was cut up into carefully separated and identified segments. Each portion was then separately subjected to artificial digestion in pepsin-hydrochloric acid-saline solution for 24 hours. This procedure did not digest away the cyst membrane and the cysts in each part of the fish were easily counted. Their distribution is shown in Fig. 8; each dot represents a cyst. The diagram is a composite one, representing the combined results of four separate fish. Most cysts are in the neighborhood of the unpaired, membranous fins (caudal, dorsal and ventral) but some are in the neighborhood of the paired fins as well. No cysts were found in the head region or in the dorsal region near the fleshy dorsal fin. All were in the actual musculature. This distribution agrees with the observations of the actual penetration of young catfish by the cercariae, which were only seen to enter by the membranous fins, never by other parts of the cuticle.

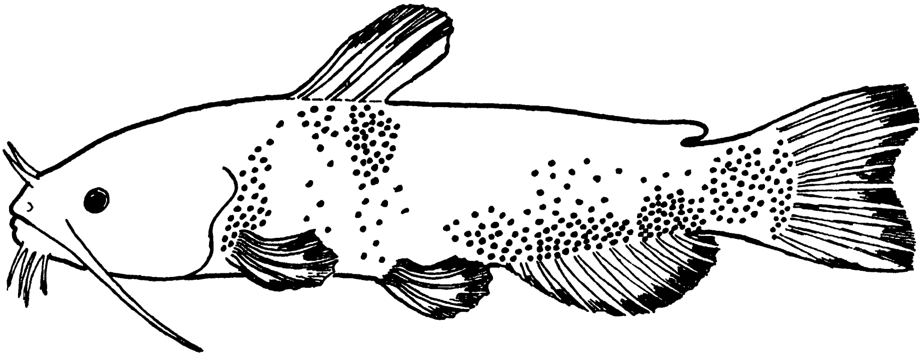


FIG. 8. *Diagram of catfish, showing distribution of metacercariae.*

Definitive Hosts

In Canada, this parasite has been found in cats, dogs and raccoons *(Procyon lotor)* as well as in the great blue heron *(Ardea herodias herodias)*. No specimens were found in the following fish-eating birds taken locally under permit from the Department of the Interior:—

8 bitterns *(Botaurus lentigenosus)*.
2 kingfishers *(Megaceryle alcyon)*.
2 gulls *(Larus delawariensis* and *L. argentatus smithsonianus)*.
3 mergansers *(Mergus merganser americanus)*.
1 hooded merganser *(Lophodytes cucullatus)*.

The development in the final host has not been systematically studied. Although artificial digestion experiments suggest that the fluke excysts only after *intestinal* digestion, Ciurea found, on autopsy, that the metacercaria of *A. donicus* after excystment became ovigerous in two and a half days. In one critical experiment carried out here however, *eggs* were not passed by the cat until the twenty-fourth day after infection, although careful fecal examinations were made daily. However, from a cat fed on experimentally infected catfish, flukes containing eggs were recovered on autopsy, a week after infection.

Several infected cats retained in the laboratory over winter had lost their infection by spring, and it seems probable that the mature life of the trematode is limited to a few months in these animals, and that the infection is maintained in the metacercarial stage in fish. This hypothesis is strengthened by the fact that no infected snails were found locally before September, although large numbers were collected and examined throughout the summer.

Careful examination of the intestines of cats passing ova, showed that the majority of the trematodes were in the ileum, a few were in the jejunum and none in the duodenum. The villi are rather far apart in the latter part of the small intestine of the cat and this may partly account for the relatively short life of the parasite.

Malformations (Plate III, Figs. 4, 5, 6)

In several cases, malformed specimens were encountered, the principal alteration being in the number of the testes. In one case both testes were completely absent, while in several one or other testis was absent. It is interesting to recall that several genera in this family have normally only a single testis, while in others the position of the testes relative to each other is a variable character. In none of the present series was the latter character observed.

Acknowledgments

During the course of this work I have had the assistance not only of my colleagues but of numerous persons outside the Institute, and I wish to acknowledge particularly the help of Mr. J. B. Harkin and Mr. Hoyes Lloyd of the National Parks of Canada, Department of the Interior, who gave us authority to take several migratory birds; Mr. L. A. Richard, Deputy Minister, and Mr. B. W. Taylor, Department of Public Works, Game and Fisheries, Quebec, who gave us authority to net fish; Mr. A. LaRocque, National Museum of Canada, who identified the snails and Professor V. C. Wynne-Edwards, McGill University, who identified the birds. I wish also to thank Professor Ciurea of Bucharest, Rumania, who gave me a number of specimens of *Rossicotrema donicum*, Dr. Price of the Bureau of Animal Industry, United States Department of Agriculture, who gave me the benefit of his experience in the systematics of this group, and Mr. J. J. Harpell, of Gardenvale, Que., who gave us permission to collect catfish and minnows from his pond.

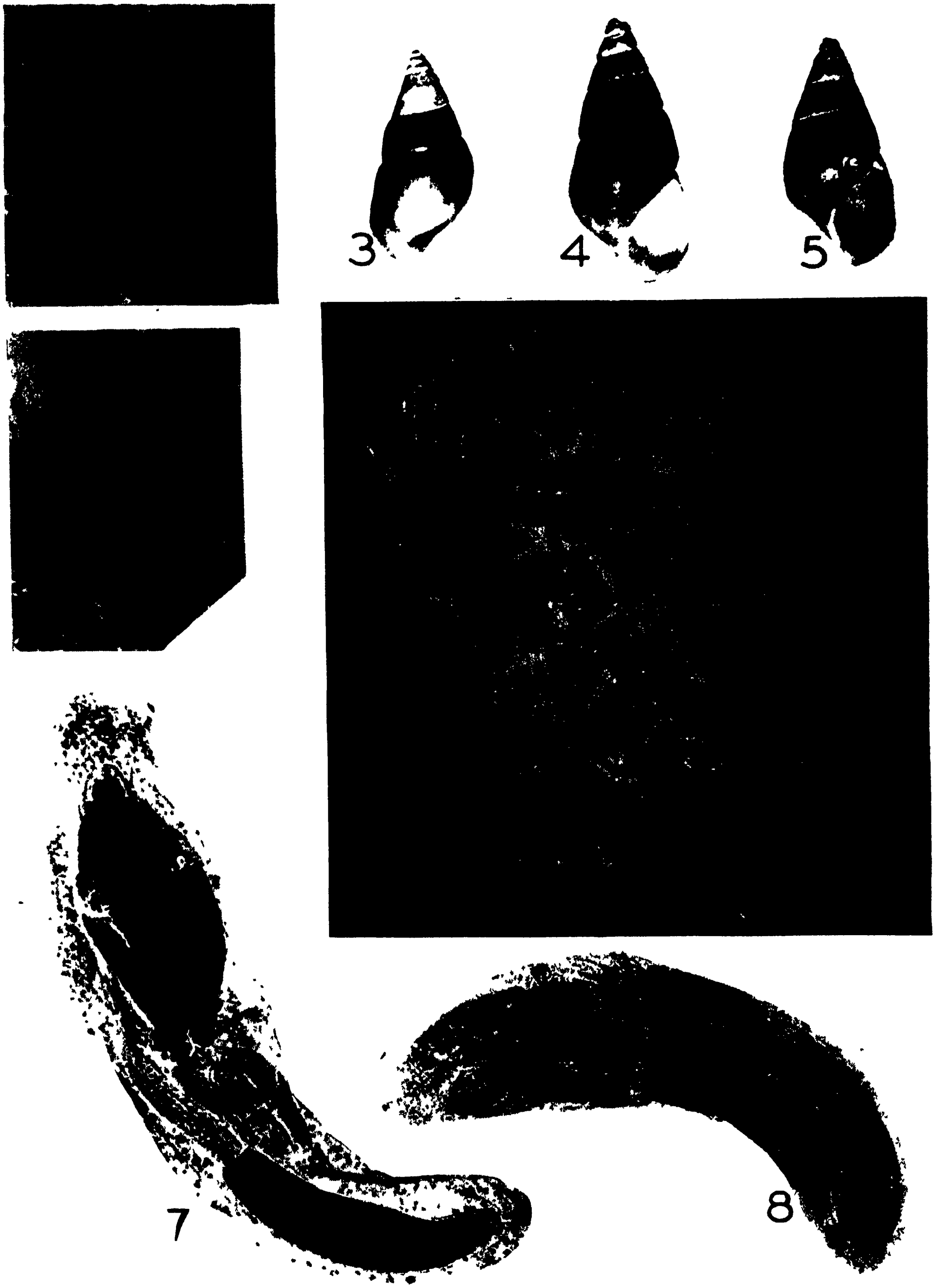
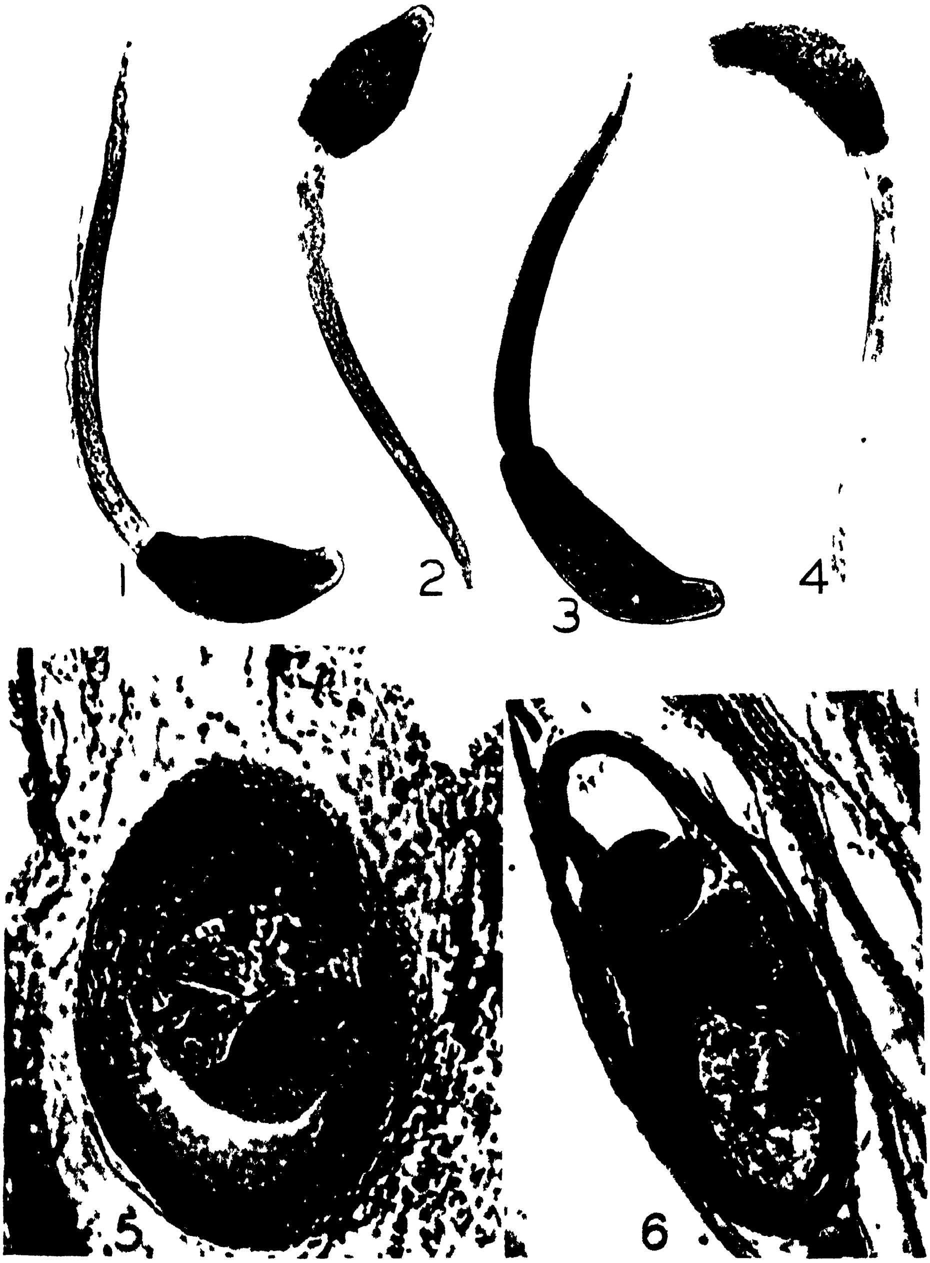


FIG. 1. *Egg of Apophallus venustus as passed in feces of cat (the operculum having been removed by pressure).* FIG. 2. *Egg containing miracidium.* FIGS. 3–5. *Goniobasis livescens.* FIG. 6. *Section of liver of Goniobasis livescens, showing daughter rediae and developing cercariae.* FIG. 7. *Daughter redia with two fully developed cercariae.* FIG. 8. *Daughter redia with eight cercariae.*



FIGS 1–4 *Cercariae of Apophallus venustus* FIG 1 *Cercaria showing flanged tail, in position of rest in water* FIG 2 *Cercaria from ventral aspect showing eyespots, glands and genital primordia* FIG 3 *Cercaria from lateral aspect* FIG 4 *Cercaria from lateral aspect showing ventral sucker* FIGS 5 6 *Sections of metacercariae in muscles of catfish (Ameiurus nebulosus)*

PLATE III

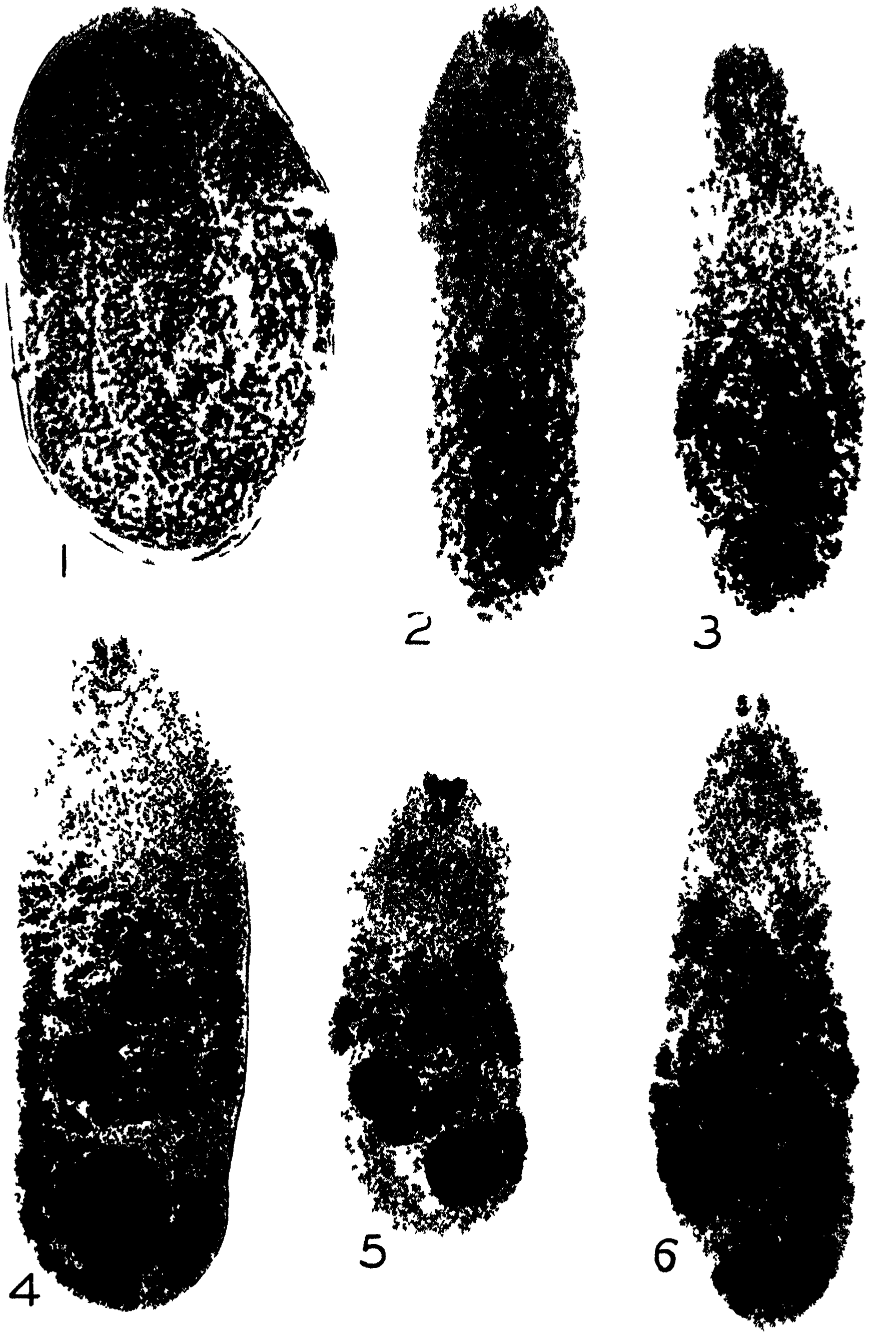


FIG 1 *Metacercaria of Apophallus venustus with fibrous cyst removed* FIGS 2–3 *Immature adults from intestine of cat* FIGS 4–6 *Malformations in adults* FIGS 4 *and* 5 *show absence of a testis and* FIG 6 *absence of both testes*

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STUDIES ON THE GENUS *RHAGOLETIS* (TRYPETIDAE) WITH SPECIAL REFERENCE TO *RHAGOLETIS POMONELLA* (WALSH)[1]

By A. D. Pickett[2]

Abstract

This paper is a report on an investigation of the biological and morphological relations of a number of species of dipterous flies belonging to the genus *Rhagoletis* (Fam. Trypetidae). If the observations made and the conclusions drawn are valid, it would appear that the practice of separating species on the basis of very slight morphological differences, which may be due to host influences, is unsound. The forms which develop in apple, hawthorn and blueberry, were studied extensively from the standpoint of their relations to their hosts and the conclusions reached are that one species *Rhagoletis pomonella* (Walsh) is involved and that the female insects show a decided preference to oviposit in the host in which they developed as larvae.

Introduction

The dipterous insect *Rhagoletis pomonella* (Walsh), variously known as the apple maggot, railroad worm and blueberry maggot, is probably indigenous to eastern North America and has been mentioned in reports of entomologists and horticulturists for many years.

There has long been considerable doubt in the minds of entomologists as to the relationships of the forms found in the different hosts in which the insect breeds. Some authors have regarded the forms that breed in apple, hawthorn and blueberry as belonging to a single species, but of late there has been a tendency to regard the blueberry form as a distinct species and in 1932 (11) it was so described.

Judging from the literature on the subject it appears to be more or less generally accepted that the apple and hawthorn forms will develop in either host and that there are no definite biological or morphological differences between them. On the other hand attempts have been made by a number of investigators to show both biological and morphological differences between the apple and hawthorn forms on the one side and the blueberry form on the other. When the present investigation was undertaken, the writer was of the opinion that some differences actually existed.

In Nova Scotia, where these investigations have been carried on, the relationships of these forms are of considerable economic importance, since in the main fruit-producing area all three host plants are found growing in close proximity and consequently, if there is a free movement from one host plant

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to another the difficulty of controlling this pest on fruits of economic importance is increased. It was with the object of clearing up the relationships of these forms that these investigations were undertaken.

As the investigations progressed it occurred to the writer that the study of other closely related forms might be of some assistance in clarifying certain points, especially those dealing with the morphology of these forms. Consequently, specimens of the form found breeding in the snowberry in British Columbia were procured and studied. This form has been described as a distinct species under the name of *Rhagoletis symphoricarpi* Curran. It has not been possible to include this form in the biological investigations, but it would be desirable to do so were sufficient living material available. The forms found breeding in cherry offer very interesting material for study and it is regretted that breeding work with these could not have been included in the present investigation as this would appear to be a problem somewhat similar to that found in the case of *pomonella.*

While it has not been possible to carry out all the breeding work that appears necessary to clear up all the points in doubt, the writer is of the opinion that the results obtained to date indicate fairly clearly what the final outcome will be. It was originally intended to include genetical studies of the forms of *Rhagoletis pomonella,* but time has not permitted this, owing to the great amount of detail involved in the breeding work.

It is hoped that the present report will renew interest in this very interesting group of insects and that it may help to clear up what were, evidently, very erroneous ideas regarding the relationships of a number of forms belonging to this genus. The biological studies are being continued.

General Considerations

In order to determine the relationships of the above mentioned group an investigational program was outlined. This was divided into two main sections: first, the morphology of the forms in question, and also of other related trypetids that were available; and second, a biological study of the forms developing in the apple, the hawthorn and the blueberry. The results of these investigations are given below.

Morphological Studies

Rhagoletis pomonella (Walsh)

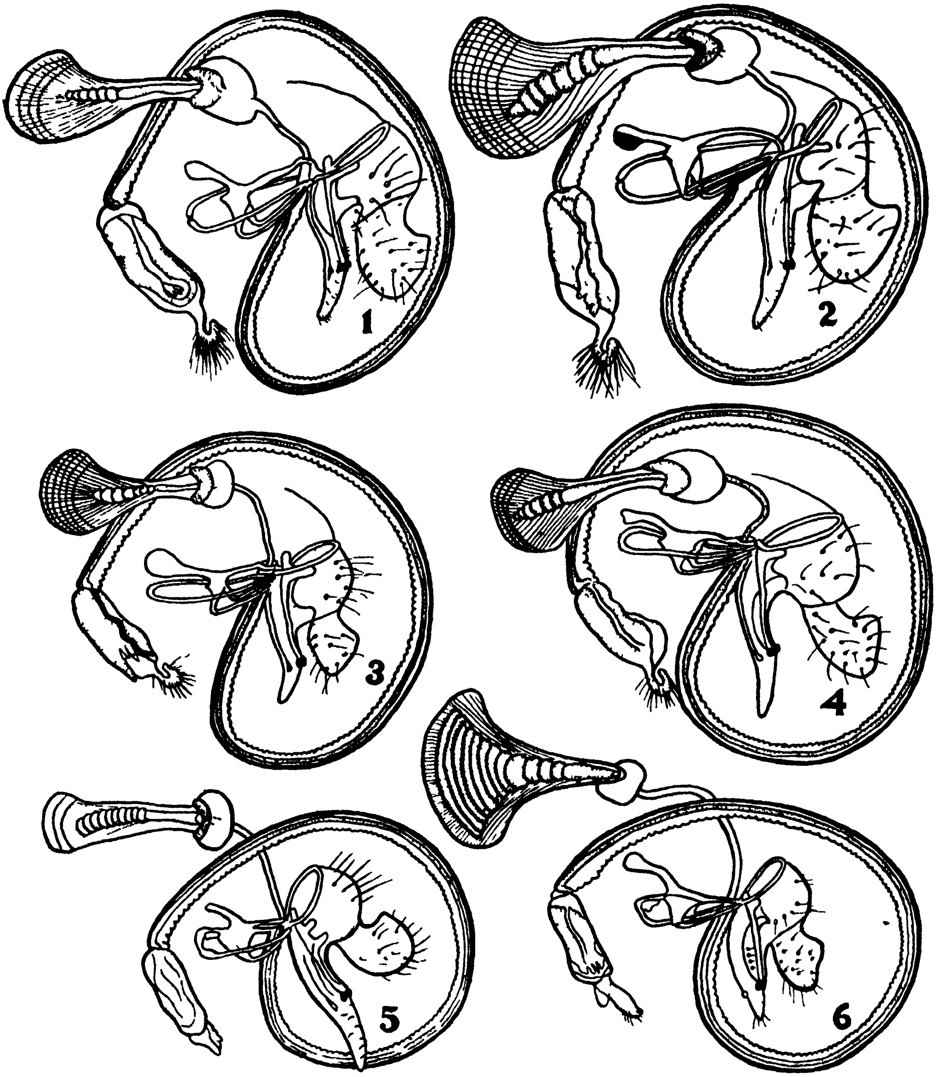
The *pomonella* group presents a difficult problem; and after a careful study of a long series of specimens, along with a painstaking survey of the literature, there is still considerable doubt as to the status of the various forms.

According to Illingworth (20), Porter (29) and Benjamin (4) the species *Rhagoletis pomonella* was described by Walsh (34) in 1867 under the genus *Trypeta;* the writer has not examined the original manuscript. This description refers to the flies developed on the apple and on the hawthorn; and there is no question as to its status. However, when Snow (31) described *zephyria* in 1894, the status of the group became somewhat complicated because the differences between the two forms were comparatively slight. Doane (12) and Aldrich (1) questioned the validity of Snow's species. Curran (9, 10), however, after an examination of one female specimen accepted *zephyria* as a definite species on the basis of color variations between it and *pomonella* (Walsh). A related form, found in the snowberry *Symphoricarpus racemosus* Michx., was described by Curran (9, 10) under the name *symphoricarpi*, on the basis of the characters of the male genitalia. Cresson (8) regards the latter as a synonym of *pomonella*. Benjamin (4) has examined one of the cotypes of *zephyria* Snow, and cannot distinguish between it and those reared from the snowberry; consequently he has designated *symphoricarpi* Curran as a synonym of *zephyria* Snow.

In 1932, Curran (11) examined specimens collected from blueberry, that were submitted to him by the writer for identification, and he described them as a new species *R. mendax*, on the basis of the characteristics of the male genitalia. Woods (35) studied the blueberry maggot in Maine, and after comparing all stages with Illingworth's (20) figures of *pomonella*, could observe no difference except in size and habits. In 1933 a series of specimens collected from apple, hawthorn, and blueberry were submitted to Dr. Alrich of the United States National Museum who, although he was unable to decide definitely (3), was of the opinion that there was not enough difference between the blueberry form and the others to give the former specific rank.

During the last four years the writer has spent a considerable amount of time studying the male genitalia of a number of species of *Rhagoletis*. A long series of the forms collected or reared from apple, hawthorn, blueberry and snowberry, have been carefully examined and a number of figures have been prepared.

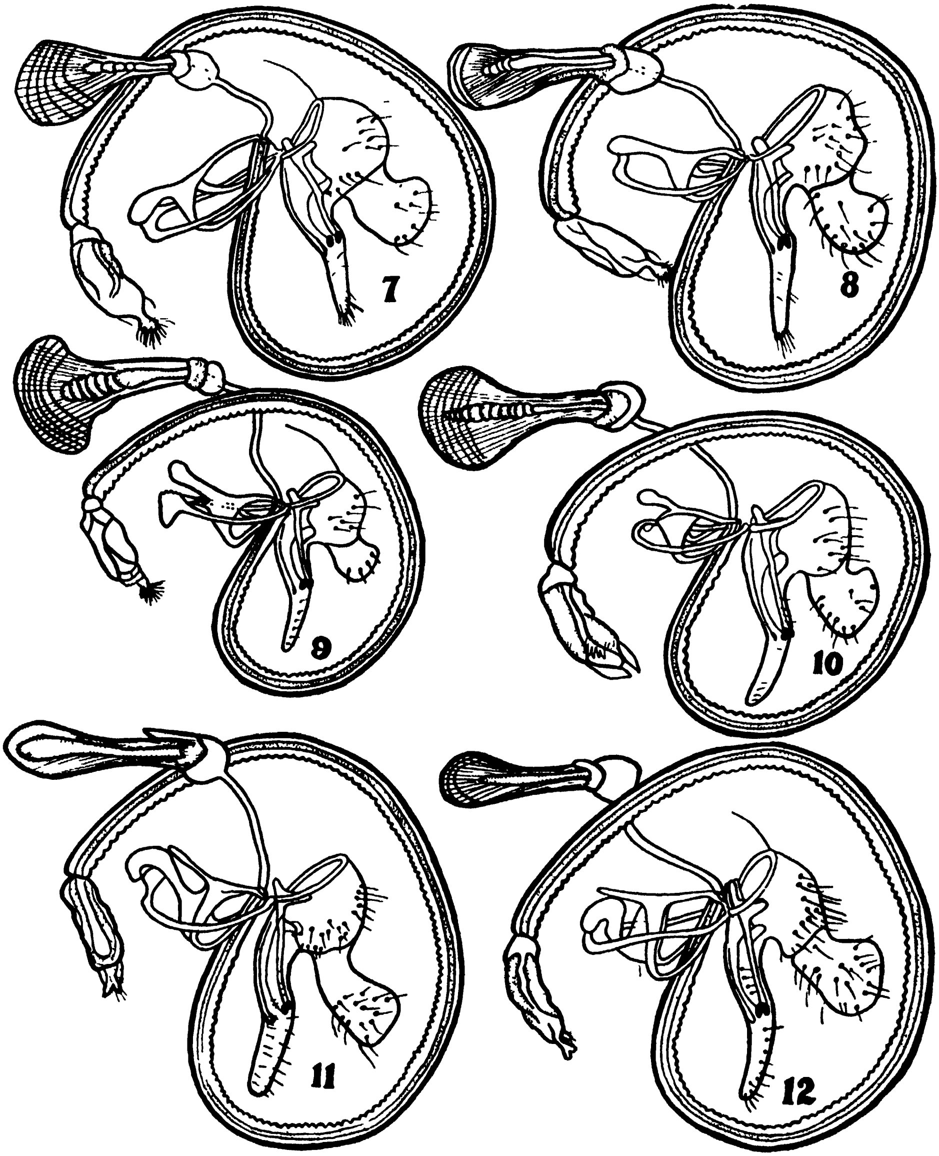
There does not appear to be any constant difference in the male genitalia of the forms that occur on apple, hawthorn and blueberry, as a reference to Figs. 1, 2 and 3 will show. Curran's figures (11) are only partially complete; and the specimens were evidently not examined in the same plane. Further, his statements that the so-called sustentacular apodeme (ejaculatory apodeme) furnishes a "ready means of determining the species" and that "this organ exhibits striking differences in shape in the various forms studied" have not been substantiated by the writer's examination of a series in each group. In the specimens examined there is a great variation in the form of the ejaculatory apodeme in each group, and this character cannot be used for the purpose of identification. The writer is of the opinion, although this point was not definitely proved, that this organ develops and becomes larger in accordance with the period elapsing after the fly leaves the puparium. Specimens of apple and blueberry flies that had just emerged showed a weak development of this organ. A careful examination of the ejaculatory apodeme of each form shows that neither the crescentic rings, the width and shape of the apical portion, nor any other feature of this organ can be used to differentiate among these forms. A brief reference to Figs. 1, 2 and 3 will show some differences in the ejaculatory apodemes. These are drawings of specimens chosen at random for this purpose and none of the slight variations are constant. It will be noted that these figures show a greater difference in the size of this organ between the flies from hawthorn and those from apple than between the apple and blueberry forms; but the difference is not constant. The observations of the writer indicate that the tip of the penis is the most constant feature found in the male genitalia of all the species of the genus studied. As Curran (11) has pointed out, the proper orientation of these structures greatly affects the conclusions that may be drawn. If mounted in liquid glycerine these organs can be readily handled. The figures show the type of apical termination and, so far as can be ascertained from a study of a series of each form, the character is constant.



FIGS. 1 to 6. *All figures of male genitalia drawn to the same scale.* FIG. 1. *Rhagoletis pomonella (Walsh) (apple form).* FIG. 2. *Rhagoletis pomonella (Walsh) (hawthorn form).* FIG. 3. *Rhagoletis pomonella (Walsh) (blueberry form).* FIG. 4. *Rhagoletis pomonella (Walsh) (snowberry form).* FIG. 5. *Rhagoletis tabellaria (Fitch).* FIG. 6. *Rhagoletis ribicola Doane.*

On a basis of the morphology of the genitalia of these three forms there are, in the opinion of the writer, no grounds for considering that more than one species is involved.

The figures shown by Benjamin (4) are different from those of Curran (10) in regard to the tip of the male clasper. The writer examined Snow's (31) figure of the wing of *zephyria* and came to the conclusion that the characters of the wing banding, on which Snow distinguished the form from *pomonella*,



FIGS. 7 to 12. *All figures of male genitalia drawn to same scale.* FIG. 7. *Rhagoletis cingulata (Loew) (cultivated cherry).* FIG. 8. *Rhagoletis cingulata (Loew) (wild cherry).* FIG. 9. *Rhagoletis berberis Curran.* FIG. 10. *Rhagoletis fausta (Osten-Sacken).* FIG. 11. *Rhagoletis suavis (Loew).* FIG. 12. *Rhagoletis completa Cresson.*

are not to be relied on. On the basis of an examination of ten specimens reared from each of the fruits, hawthorn, apple and blueberry, the hyaline indentation between crossbands two and three reach the fourth vein in some of each of these forms. In those bred on hawthorn, one specimen out of ten exhibited this condition; in the apple flies four specimens out of ten; and in the blueberry flies seven specimens out of ten showed this character.

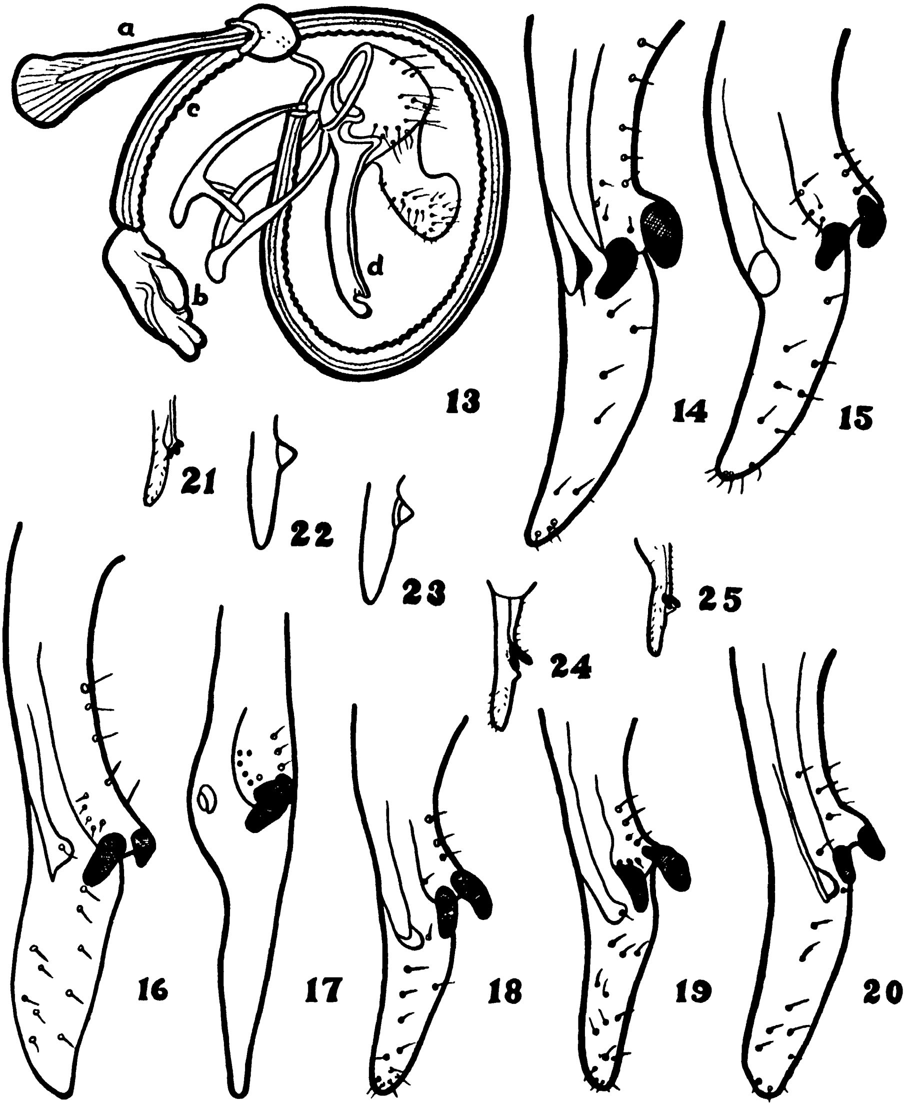
The situation regarding the form from the snowberry is slightly different. The writer has not had an opportunity to examine the form reared from the sparkleberry *Batodendron arboreum* Marsh (Nutt) which Benjamin (4) considers to be conspecific with the snowberry form and also with Snow's *zephyria.*

Curran (11), in reaffirming his identification of *symphoricarpi* as a species, states that in his examination of the male genitalia he probably overlooked the most striking differences. Benjamin (5) maintains that the differences in the male genitalia as referred to by Curran (10) are sufficiently constant to separate this form from *pomonella* (Walsh).

The writer has examined the genitalia of a long series of each form and although certain characters mentioned by Curran (10) are fairly constant, even though somewhat variable, he cannot agree that the differences mentioned in Curran's (11) key are constant. Curran's (10) figures, however, are essentially correct in so far as they show the general shape of the claspers; but the statements regarding the lobe of the clasper and the length and shape of the hooks are not borne out by the present studies (Figs. 16–25). In most specimens, however, it is possible with careful orientation to see the more curved condition of the clasper in the apple form; the more ventral position of the apical tip or point of the clasper as compared with the form on the snowberry; and the wider and more compressed condition of the clasper beyond the spurs as seen in the latter. It is necessary to study these forms very carefully to note these apparent differences, as a slight variation in position during the examination entirely changes the appearance of these parts.

If one studied only the forms found on apple and snowberry it would be fairly easy to come to the conclusion that these differences were of sufficient significance and consistency to separate the forms as different species; but when the forms reared on hawthorn and blueberry are considered, some doubt develops. Some of the male hawthorn flies show an even greater divergence from those of the snowberry than do the apple flies. That is, the differences referred to above appear to be somewhat more intense (Figs. 14–16). This does not hold true for all individuals. When forms from the blueberry are studied, certain individuals, probably the minority, show these characters as being nearly midway between the apple and snowberry forms. These characters may be noted by comparing Figs. 14–25. With this additional information, the writer is inclined to believe that the snowberry form should not be regarded as a separate species.

One other feature which exhibits the very close relationship among all these forms is the nature of the tip of the penis, in which there is absolutely no variation so far as can be found when complete specimens are examined. Among all the well defined species studied by the writer in this genus, with one possible exception, which will be referred to later, there were distinct differences in this part of this organ.



FIGS. 13 to 25. FIG. 13. *Male genitalia of Epochra canadensis Loew. a. Ejaculatory apodeme. b. penis. c. penis tube. d. claspers.* FIGS. 14–25. *Claspers of Rhagoletis pomonella (Walsh).* FIG. 14. *Right clasper of hawthorn form (latero-medial aspect).* FIG. 15. *Right clasper of apple form (latero-medial aspect).* FIG. 16. *Right clasper of snowberry form (latero-medial aspect).* FIG. 17. *Postero-lateral aspect of clasper shown in Fig. 16.* FIGS. 18–20. *Right claspers of blueberry form showing variations in apex.* FIG. 21. *Clasper of apple form showing three hooks.* FIG. 22. *Clasper of apple form showing shape of lobe opposite hooks.* FIG. 23. *Clasper of snowberry form showing shape of lobe opposite hooks.* FIG. 24. *Posterior aspect of clasper of apple form showing hooks and lobe.* FIG. 25. *Posterior aspect of clasper of snowberry form showing hooks and lobe.*

A study of the larval characters which are mentioned by Greene (17) in his study of trypetid larvae was completed. This failed to indicate any significant differences between maggots taken from hawthorn, apple, blueberry and snowberry.

The specimens from snowberry were supplied by Mr. W. Downes, Victoria, B.C.

Rhagoletis cingulata (LOEW)

In the past this has been considered a very stable species with clearly defined characters, but in 1932 Curran (11) named two new species from closely related forms. The writer obtained specimens of both of these and dissected the male genitalia for comparison with the form reared from cultivated cherries. After comparing a series of the form which Curran (11) has designated as *indifferens* with a series of *cingulata* there is no doubt in the mind of the writer that the two are identical in so far as the male genitalia are concerned, and that *indifferens* should be considered as a synonym of *cingulata*. The differences mentioned by Curran (11) do not hold; and here again it may be pointed out that the size and shape of the ejaculatory apodeme is so variable in all these forms that it cannot be used as a taxonomic character. Reference to Figs. 7 and 8 will show that in the two forms figured, the form designated as *indifferens* by Curran has a somewhat wider ejaculatory apodeme than has the form from the cultivated cherry. This feature is not constant, however, and varies greatly in the specimens studied. The other differences mentioned, but not specified by Curran (11), were not observed by the writer. It will be noted also by the figures that the tip of the penis, which appears to be a fairly good taxonomic character, is similar in both forms. The findings as cited above agree with the observations of Benjamin (4) on forms reared in Florida.

The specimens of *cingulata* studied were supplied by Prof. L. Caesar, Guelph, Ontario, and Dr. F. L. Gambrell, Geneva, N.Y. The western form from the wild cherry was supplied by Mr. S. C. Jones, Corvallis, Oregon.

Rhagoletis berberis CURRAN

This is a new species established by Curran (11) on the basis of the nature of the wing banding. The wing pattern figured by Curran (11) appears constant in the specimens examined by the writer. These were from the type locality. Further, an examination of the male genitalia indicates that the species is distinct from *cingulata*. Figs. 7–9 show a number of differences, but the writer considers the nature of the tip of the penis to be of particular significance.

Curran (11) has mentioned the resemblances of this species to *R. completa* Cresson, and there does appear to be a fairly close relationship in both wing banding and male genitalia. The writer, however, is of the opinion that, as the differences found in the latter, as shown in the figures, are fairly constant and as the general body color is much darker in *berberis*, they should be regarded as distinct species.

Curran's types were reared from the fruits of *Berberis nervosa* at Hood River, Oregon, by Mr. S. C. Jones. The writer's specimens were supplied by Mr. Jones, but are labelled as being from grape.

Rhagoletis fausta (OSTEN-SACKEN)

There appears to have been very little confusion about the status of this species since Aldrich (2) established the synonomy of *R. intrudens* Aldrich. A good description of the species is given by Cresson (8) but the male genitalia have not been figured previously so far as can be ascertained. Fig. 10 shows that the male genitalia of this species are quite different from those of the other species studied, which indicates the value of these characters in a taxonomic study of the genus.

Specimens were obtained from Dr. F. L. Gambrell, Geneva, N.Y.

Rhagoletis sauvis (LOEW) AND *R. completa* CRESSON

Rhagoletis sauvis was described by Loew (22) in 1862; and in 1929 Cresson (8) established *completa* as a subspecies. Boyce (5) considers the differences great enough to establish the latter as a distinct species. He figures two characters found in the male genitalia, the validity of which the writer must question. It is true that there are slight differences in the claspers, but these are not as pronounced as shown by Boyce (5) and it would appear that a different perspective was used in each case. Boyce's figure of *completa* compares favorably with Fig. 12, but to get the claspers of *sauvis* to appear like those in Boyce's figure, it is necessary to orientate the part so that a postero-lateral view is obtained. Fig. 11 shows the condition observed by the writer. Further, these studies showed no constant difference in the size or shape of the ejaculatory apodeme, and as pointed out previously in connection with other species, this character cannot be relied upon in taxonomic determinations. A comparison of Figs. 11 and 12 shows these organs as they are found in the two specimens chosen at random for drawing. The distinguishing characters, as illustrated by Boyce (5) may occur in some specimens in either form, but cannot be considered as constant.

A reference to the figures will show the very close relation of the two forms. The differences between these are certainly not marked, the character of the wing pattern being probably the most pronounced. It was not possible to find any distinct differences in the tip of the penis. There may be sufficient justification for establishing *completa* as a subspecies, but in the opinion of the writer there is not sufficient evidence of differences to consider that two distinct species are involved. However, in order to establish definitely the status of those forms, genetical and cross-breeding investigations should be undertaken.

The specimens of *sauvis* were obtained from Dr. F. L. Gambrell, Geneva, New York, and those of *completa* from Dr. A. N. Boyce, Riverside, California.

Rhagoletis tabellaria (FITCH) AND *R. ribicola* DOANE

Rhagoletis tabellaria was described by Fitch (13) in 1856 under the genus *Tephritis*. In 1898, Doane (12) described a closely related form found on currant and gooseberry in Washington as *R. ribicola*. Aldrich (1) recognized this as a valid species and figured the wing. In 1915, Marcovitch (23) described a new species which he called *juniperinus* from flies reared on *Juniperus virginiana* in New York state. This was accepted by Phillips (27) as a valid species. The writer has not had an opportunity of studying the latter form, but the wing pattern as figured by Marcovitch (23) and Phillips (27) appears to resemble the form *ribicola* rather than *tabellaria*. Cresson (8) regards *ribicola* and *juniperinus* as synonyms of *tabellaria*. The writer has dissected and studied the male genitalia of one specimen of *ribicola* from Washington and considers it to be quite distinct from the specimen of *tabellaria* studied. Figs. 5 and 6 indicate very distinct differences between these species in regard to the shape and length of the claspers and also the nature of the tip of the penis. It will be noted that there are marked differences in the size and shape of the ejaculatory apodeme in the two figures, but it would require a study of a long series of specimens to determine the value of this character, and these are not available. However, there are such wide variations in these organs in all species where a series was available for study, that this character should be disregarded, at least until proved to be of value. On the basis of this examination the writer considers that *tabellaria* (Fitch) and *ribicola* Doane should both be regarded as distinct species.

Specimens examined were as follows: One specimen of *tabellaria* and one of *ribicola* from Mr. W. J. Brown, Entomological Branch, Ottawa; one specimen of *ribicola* from Mr. J. F. Gates Clarke, Pullman, Washington; one specimen of *tabellaria* from Dr. E. A. Chapin, United States National Museum, Washington, D.C.

Biological Studies

HISTORICAL

Walsh (34) described *Rhagoletis pomonella* from specimens reared from apple and hawthorn and therefore, inferentially, he regarded them as being identical. Other authors, so far as can be determined from the literature, have not considered there were differences between the flies that pass their larval stages in the apple and those found in haws.

In addition to the apple and hawthorn, crab apple, pear and plum have been reported as hosts; and Lathrop and Nickels (21) reared this species from the following plants found on blueberry barrens:

Blueberries: *Vaccinium angustifolium* Kalm (m.a.), *V. canadense* Ait. (m.a.), *V. corymbosum* L. (m.a.)

Bunchberry, *Cornus canadensis* L. (m.a.)

Chokeberry, *Aronia melanocarpa* (Michx.) Britton (m)

Huckleberry, *Gaylussacia baccata* (Wang) C. Koch (m.a.)
Mountain cranberry, *Vaccinium vitisidaea* minus Lodd (m)
Dwarf serviceberry, *Amelanchier bartramiana* Roem. (m)
Wintergreen, *Gaultheria procumbens* L. (m)

(m) indicates fruits in which maggots were found, presumably *R. pomonella.*
(a) indicates species from which adults were reared.

Whether these flies would change from one host to another has not been studied until recently, although several authors record observations on this point.

Illingsworth (20) published a detailed account of investigational work on *R. pomonella* carried on at Cornell University in 1911 and 1912. He also gives a technical description of all stages of the insect and records data on distribution and host plants. O'Kane (25) gave comprehensive historical and distributional reports, as well as information on the known hosts.

Woods (35) succeeded in successfully transferring very small larvae from huckleberries and blueberries to the fruit of the chokeberry, *Pyrus melanocarpa*, with the result that these individuals completed their development and pupated. He further observed that only in certain localized areas were blueberries infested with this insect, and that in many cases blueberries growing in the near vicinity of severely infested apple orchards showed no trace of the insect.

The same writer (35) failed to induce apple flies to oviposit in blueberries by confining them in cages on blueberries both in the field and laboratory. Attempts were made to transfer half-grown blueberry maggots to apples by inserting them beneath the skin; but these failed to develop. An attempt to induce flies taken on the blueberry to oviposit in apples also proved abortive. He does not state whether he succeeded in getting flies reared on any fruit to oviposit in captivity on that fruit. Wood's (35) conclusion is quoted as follows: "At any rate, the writer is inclined very strongly to believe that biologically at least there are two distinct strains or races of *Rhagoletis pomonella* Walsh, the one breeding in the apple and related fruits and the other in small fruits such as the blueberry and huckleberry. There does not seem to the writer to be any other conclusion which will explain the data given above. Certainly in so far as *Rhagoletis* occurs in Maine, the form on apple and the form on the blueberry are entirely independent." Similar observations and conclusions are recorded by Patch and Woods (26).

Porter (29) says, "Whether the flies which infest the different fruits are all of the same species is open to serious question. The occurrence of the species in fruit of hawthorn in localities in which the apple is free or virtually free from attack, the reverse condition in other localities, the presence of maggots in blueberries in certain restricted areas and in huckleberries in others, and the distinctly different habits of the blueberry flies from the flies in the apple orchard, all point to the possibility that there may be several distinct species, biological races, or incipient species, which at present cannot be distinguished from one another."

According to Porter (29) the earlier investigators found difficulty in inducing flies to oviposit in cages.

Lathrop and Nickels (21) encountered difficulty in rearing specimens in confinement and, therefore, were unable to carry on cross breeding and other experiments to procure biological data on the relationship of the flies reared on different hosts. They were able to transfer first instar blueberry maggots to the apple and have them develop. From 200 second instar blueberry maggots treated as above, about 20 developed to the pupal stage and one adult was obtained. These were normal in size for the blueberry form. Second instar apple maggots were transferred to blueberries and some of these formed puparia but no adults emerged. The puparia were normal in size for the apple form. They also state that each maggot consumed two or more blueberries during its development. Their concluding paragraph is quoted, as follows: "It seems probable that the blueberry maggot and the apple maggot exhibit an example of incipient species formation, and from an ecological viewpoint the two forms seem distinct and independent."

In 1931, Fluke and Allen (14) reported on an investigation carried on in Wisconsin in which they secured ready mating and oviposition in cages by feeding a mixture of 1–3% yeast in 5% honey water. This greatly facilitated the carrying out of investigations on these insects.

McAlister and Anderson (24) working on the blueberry maggot in Maine used a modification of Fluke and Allen's method. They carried on interbreeding experiments and found mating occurring when virgin females reared on apple were caged with males reared on the blueberry. However, copulation was less frequent when reciprocal crosses were made. No maggots developed from the latter cross, but of the ten apple females used in crosses with blueberry males, seven deposited eggs in blueberries from which 17 maggots hatched, 15 of which matured and pupated.

METHODS USED IN BREEDING WORK

During the first two years a fairly satisfactory technique for handling the flies was worked out. This is described as follows:

In the summer, heavily infested fields of blueberries were located and about the time the maggots began to emerge in numbers large quantities of berries were picked for the purpose of obtaining flies for the following season's investigations. The berries, in some cases, were spread out over the ground to a depth of one to two inches in a sheltered location and the maggots emerged from these and entered the soil to pupate. In other cases the berries were placed on soil in flats made with wire screen bottoms. These were set in the ground so that conditions would be as nearly normal as possible. When virgin flies were needed for crossing experiments, the flats were removed to the insectary the following June; but when they were used in studying host relations, wire cages, constructed in a four-sided dome shape and covered with black cloth, were placed over the soil or flats in which the puparia were located. On the top of the cage was placed a small removable wire screen cage. The flies were allowed to enter this as they emerged from the soil, and a shutter made of a thin piece of board or shingle was arranged so that the passageway between the large and small cages could be closed. This allowed for the removal of the small wire cage so that the flies could be taken from it, or so that it could be replaced by another without the loss of the flies.

In the rearing of apple and hawthorn flies, heavily infested fruit was located in the autumn. When the majority of the maggots were nearing maturity the fruit was gathered and taken to the insectary. Here it was placed in boxes with coarse wire bottoms that would keep the fruit in, but would allow the maggots to pass through freely. Underneath these boxes were placed flats filled with ordinary fine garden soil. As soon as all the maggots had emerged from the fruit and entered the soil, the flats were set outdoors in the earth in a sheltered place, and left until the following June.

Just before it was time for the flies to emerge, the pupae were washed out of the soil by floating them in water. They were then put in sand, in wire cages made about 6 in. in diameter and 8 in. high with wooden tops and bottoms. These cages were covered with dark paper, and a ¾ in. hole was made in the centre of the top of each cage. A paper cone leading up to this, inside the cage, directed the flies toward the opening. A glass tube connected this cage with a small wire cage about 3 in. by 3 in., constructed exactly as the larger ones, except that the wire screening was not covered. When the flies emerged they were attracted to the light, and consequently crawled up into the smaller cage. These small cages were made with a ⅜ in. glass tube in the top through which the flies might be fed. The flies were kept in the small cage until there were enough to take to the field, then the cage was removed and replaced by another.

Before any flies had emerged, clusters of fruits of susceptible varieties located in suitable places were selected, and breeding cages placed over them. The breeding cages were made of 20-mesh japanned wire screening. A circular piece of pine about ¾ in. thick formed one end. In this two holes were bored, one ¾ in. in diameter for putting the flies into the cage, and the other, through which the flies were fed, was ⅜ in. to hold a glass tube long enough to reach to the inside of the end and project about 1 in. on the outside. To the other end was attached a cotton sleeve. In apples and hawthorns, the end of the limb that bore the fruit to be covered was cut off just beyond the fruit, or in the case of a smaller limb, it was doubled back and the cage pulled up over the fruit and the cotton sleeve attached tightly to the limb above it. Usually another limb was tied to the first to prevent swaying and subsequent damage to the cage. The cages were placed so that they were fairly well protected and not exposed to too much direct sunlight. With the cages on blueberries great care had to be exercised in regard to the latter point. The cages were so close to the ground and the air moved through them so slowly, owing to the protection afforded by the dense, low-growing vegetation, that a heavy mortality occurred during the bright warm days unless sufficient shade was provided.

When a sufficient number of flies were procured from the emergence cages, or from field collections, they were taken to the field cages in vials or in the small cages described above. In any case, the flies were put into the cage through the large hole in the end. The cages were connected by a piece of glass tubing about 1½ in. long and of the proper diameter to fit into the hole in the end of each. The small cage was then covered with black paper or cloth and the flies moved toward the light, to enter the larger cage. When they were all in, the small cage was removed and the opening in the breeding cage stoppered. The stopper could be easily removed to permit the addition of more flies. Although this method of transferring flies to the field cages was fairly satisfactory, it proved very slow at times, and in many cases the cotton sleeve was loosened and the small cage placed inside and left until feeding time the following day.

During the first two years of the investigations the flies were fed according to Fluke and Allen's (14) formula. In 1933, however, the writer was informed by Mr. James Marshall, Assistant Entomologist, State College of Agriculture, Washington, that Dr. L. B. Ripley (30), working on the Natal fruit fly in South Africa, had used cow's milk along with the Fluke and Allen mixture and had obtained excellent results. Dr. Ripley's letter to Mr. Marshall is quoted in part as follows: "The Fluke-Allen food does not develop eggs in our females and mating very rarely occurs on this diet, but when it is made with 50% milk, egg production is much increased and mating fairly frequent in large cages."

As this information was originally given to the writer verbally, he was under the impression that the percentage of milk was 5% instead of the 50% quoted above. However, the 5% mixture was used with fairly satisfactory results, although the stronger mixture might have been better. The mixture used was 5% honey, 5% milk and 2 to 3% yeast in distilled water. The mixture was made up fresh before each feeding and the feeding was ordinarily done quite early in the day, usually between eight and ten o'clock, as it was observed that the flies appeared to feed more at that time. Small pieces of absorbent cotton soaked in the feeding mixture were inserted into the glass tubes in the ends of the cages. New cotton was used at each feeding, that used the previous day being removed and discarded.

In securing virgin flies for cross-mating experiments, the methods described above were followed until the time the pupae were washed from the soil. In order to isolate each individual, ⅝ in. holes were bored in pieces of 2-in. planed hardwood planks about 4 ft. long. The holes were placed about ½ in. apart each way so that there were several hundred in each plank. Two or three thicknesses of blotting paper, cut to fit, were placed in the bottom of each hole. The pupae were placed one in each cell and a piece of fine wire screening was placed over the top of each and held in place by two tacks.

The planks were covered with damp newspapers to hold the moisture and then stacked up in the insectary. Each morning all flies that had emerged during the previous 24 hr. were removed and the cells containing pupae were moistened by a few drops of distilled water inserted with a medicine dropper. As the flies were removed they were examined and sorted according to sex and host plants and then placed in small wire cages like those described above, and fed. They were kept in the insectary until a sufficient number of each group had been accumulated to start a cage, when they were transferred to the field cages.

In the cross-breeding experiments the females were put in cages on the host on which they developed as larvae. It was thought that if mating did occur the females would be more likely to oviposit in these than in a fruit of some other species. Where males for the crosses were not available from reared material, they were collected from their host plants.

Observations were made each day on the flies in the cages and records kept of any data of interest such as the behavior, copulation, egg-laying, and so forth.

When the flies were all dead, or when there was evidence that the fruit was likely to deteriorate rapidly, the cages were removed and the fruit either deposited over earth in wire cages, or placed in glass vials covered with cheesecloth. When all the maggots had left the fruit, the earth was sifted and the pupae counted. Later they were placed in earth and put in a protected place out of doors for observation the following year.

The variety of apple used in the 1934 and 1935 investigations was the Bough Sweet. This is a soft, early, sweet variety, which is very susceptible to apple maggot attacks. Gravenstein was also used in the earlier investigations, but this variety is not as susceptible as Bough Sweet.

BREEDING INVESTIGATIONS (1931–32–33)

At the time these investigations were initiated there was no definite information regarding the biological relation of the flies developing on the apple, hawthorn and blueberry. In 1931, experiments were undertaken to find out whether the flies which had developed during their larval period in the fruits of one host could be induced to oviposit in another host, and if oviposition did take place, whether larvae would develop in the new hosts.

Since it was generally considered that the apple and hawthorn flies would cross from one host to the other fairly freely, the first experiments were to ascertain whether the blueberry fly would oviposit and develop in apple; this being the more important economic problem in Nova Scotia.

The breeding work undertaken may be divided into two divisions: First, to find out whether flies reared on one host would oviposit on another; and second, to determine whether flies reared on different hosts would interbreed.

TRANSFERRING FROM ONE HOST TO ANOTHER

At Morristown, in 1931, a small Cox Orange apple tree, a variety susceptible to apple maggot attack, was entirely covered with a cheesecloth cage made as tight as possible. It was felt that if a large cage were used the flies would be more contented and more likely to oviposit. A large number of flies, approximately one hundred, consisting of both sexes, were gathered from blueberry bushes in an adjacent pasture and liberated in the cage. No attempt was made to feed these flies, as it was thought they would be able to find sufficient food in such a large cage (approximately 12 × 12 × 10 ft.). Although frequent examinations of the tree were made, none of the flies were ever observed and it was assumed that they either escaped or died. On trees nearby, blueberry flies were confined in cages similar to those described by Fluke and Allen (14) and fed on the yeast-honey-water mixture suggested by these workers. The flies were fed daily and lived on the average from ten days to two weeks. None was ever observed to make any attempt to oviposit and the flies seemed restless in the cages. When the cages were removed no stings were visible on the apples and no maggots were found in the fruits.

In 1932, more extensive trials were carried out. Before any flies emerged, cheesecloth bags were placed over fruits of hawthorns, blueberries, and susceptible varieties of apples. When flies began to appear on these fruits in locations where they were plentiful, they were captured in fairly large numbers and placed in cages on the various hosts mentioned; that is, flies collected from each host plant were confined in cages placed on apple, hawthorn and blueberry. During this year difficulty was encountered in keeping the flies alive for any length of time. Some difficulty was experienced at first in obtaining wire netting fine enough to hold the smaller flies collected from the blueberry, the wire used first being ordinary 14-mesh japanned wire screening. When 20 mesh copper wire was used, the flies did not appear to live a normal period and it was thought that the copper must be toxic to them. Finally, a suitable type of wire was procured, but it was then too late in the season for it to be of value. Difficulty was also experienced, especially in the cages on blueberry, in supplying the proper amount of shade. If the cages were too much exposed to the sun, a very warm day with bright sunshine would produce a high mortality. Similar observations have been reported by McAlister and Anderson (24). However, in spite of the difficulties encountered, the flies in cages on the host in which they developed as larvae appeared quite contented. Oviposition occurred, maggots developed, and puparia were formed. In only one did any crossing occur and that was in the case of the apple fly on hawthorn; and in one of these cages one maggot developed and formed a puparium.

The work carried on in 1933 showed, largely, a repetition of the 1932 results. The flies behaved normally in cages on the host plants in which they developed as larvae but no crossing occurred, and it appeared that if crossing did occur, it was so rare that it might be regarded as accidental. However, the economic importance of the problem was so great that it was decided to carry on the work another year.

RESULTS OF BREEDING WORK (1934–35)

Table I shows the results obtained in the 1934 investigations. It is to be regretted that the data recorded were lacking in some respects, but it might be said also that data of uncertain validity were discarded.

It will be noted that eight of the 12 cages gave negative results. Cage 1 contained flies collected in the adult stage from the leaves of the apple. These flies seemed to behave normally and the apples were heavily marked by egg-laying punctures. It will be noted that 77 pupae were obtained.

Cage 2 contained flies that had developed as larvae in apples and had emerged from puparia in the insectary and were then transferred to the orchard. Why there was no oviposition here cannot be explained. It might be contended that these flies which emerged in the insectary were not as virile as those collected from the trees, but other data do not substantiate this, as the female apple flies used in Cage 3 also emerged in the insectary. In Cage 3 were apple females and blueberry males, and 27 pupae were obtained. This confirms the results of the work conducted in Maine by McAlister and Anderson (24). These flies appeared to behave in a normal way and lived a fairly long time, mating being noted on several occasions. No results were obtained in cages where blueberry or hawthorn flies were placed on the apple.

Blueberry flies collected from the bushes oviposited in Cage 8, and 21 pupae were obtained. Blueberry flies that had emerged in the insectary showed negative results in Cage 9, but the number of flies involved here was small. In Cage 12, apple flies collected from the trees oviposited in blueberries, and six pupae were obtained. The flies in this cage appeared quite contented after being caged for a few days and they were frequently seen feeding on the surface of the berries. The number of pupae collected appeared small compared to the number of berries apparently infested; it is felt that a number of larvae escaped before pupation, as three were found outside the cage. The report of McAlister and Anderson (24) is hereby confirmed, since it was the female apple flies which they reported as ovipositing in blueberries.

There is some doubt in regard to the results of the work on hawthorn, since the fruits were not covered early enough to preclude all possibilities of their being infested. They may therefore be disregarded.

No adults emerged from the cross-bred puparia that were kept over the winter, but this is not regarded as significant, as those reared on their own hosts also failed to emerge. It is thought that environmental conditions during the winter may not have been normal.

Table II shows the results obtained in 1935. It will be seen that information on Cages 28 and 29 is missing; this is due to the unreliability of the data. Some points of interest are as follows: In Cage 10, on blueberry, apple flies collected from the trees gave one pupa. Similar flies on blueberries in Cage 11 produced two pupae. All the crosses on blueberry gave negative results, and flies reared on the hawthorn also failed to develop on blueberry.

TABLE I

Cage No.	Host	Host in which reared		Place of emergence	No. of flies	Noted		Date started	No. of pupae
		Females	Males			Mating	Stings		
1	Apple	Apple	Apple	Apple trees	—	—	Yes	Aug. 9	77
2	Apple	Apple	Apple	Insectary	—	—	No	—	0
3	Apple	Apple	Blueberry	Insectary (females)	—	Yes	Yes	—	27
4	Apple	Apple	Hawthorn	Insectary (females)	Small	—	No	—	0
5	Apple	Blueberry	Blueberry	Collecting cages	—	—	—	—	0
6	Apple	Blueberry	Blueberry	Bushes	—	—	No	—	0
7	Apple	Hawthorn	Hawthorn	Bushes	—	—	No	—	0
8	Blueberry	Blueberry	Blueberry	Bushes	24	—	—	—	21
9	Blueberry	Blueberry	Blueberry	Insectary	Small	—	No	—	0
10	Blueberry	Blueberry	Apple	Insectary	5	—	No	—	0
11	Blueberry	Hawthorn	Hawthorn	Bushes	—	Yes	No	—	0
12	Blueberry	Apple	Apple	Apple trees	—	—	—	Aug. 9	6

TABLE II

Cage No.	Host	Host in which reared		Place of emergence	No. of flies	Noted		Date started	Date removed	No. of pupae
		Females	Males			Mating	Stings			
1	Blueberry	Blueberry	Blueberry	Collecting cages	92	Yes	No	July 15	Aug. 16	0
4 and 4a	Blueberry	Apple	Apple	Insectary	200	No	Yes	July 20	Aug. 20	0
7	Blueberry	Blueberry	Apple	Insectary	62	Yes	No	July 20	Aug. 19	0
8	Blueberry	Blueberry	Blueberry	Collecting cages	49	—	—	July 22	Aug. 19	11
10	Blueberry	Apple	Apple	Apple trees	35	—	Yes	July 24	Aug. 19	1
11	Blueberry	Apple	Apple	Apple trees	44	—	Yes	July 24	Aug. 19	2
13	Blueberry	Hawthorn	Hawthorn	Insectary	114	—	Yes	July 26	Aug. —	0
16	Blueberry	Blueberry	Hawthorn	Insectary	5	—	—	Aug. 1	Aug. 19	0
17	Blueberry	Hawthorn	Hawthorn	Bushes	16	—	—	Aug. 1	Aug. 19	0
31	Blueberry	Hawthorn	Hawthorn	Insectary	80	—	—	Aug. 2	Aug. 24	0
35	Blueberry	Blueberry	Blueberry	Bushes	34	—	—	Aug. 13	Aug. 20	0
2	Apple	Blueberry	Blueberry	Collecting cages	199	Yes	Yes	July 18	Aug. 20	0
3	Apple	Blueberry	Blueberry	Insectary	181	Yes	Yes	July 20	Sept. 4	0
5	Apple	Apple	Blueberry	Insectary	122	Yes	Yes	July 20	Aug. 28	26
6	Apple	Apple	Apple	Insectary	170	Yes	Yes	July 20	Aug. 20	42
9	Apple	Apple	Apple	Apple trees	20	Yes	Yes	July 24	Aug. 20	16
12	Apple	Blueberry	Blueberry	Bushes	52	—	Yes	July 25	Aug. 28	14
15	Apple	Apple	Hawthorn	Insectary	30	Yes	Yes	Aug. 1	Sept. 13	0 (1 dead larva)
33	Apple	Hawthorn	Hawthorn	Insectary	170	—	Yes	Aug. 5	Sept. 2	17
20	Hawthorn	Apple	Apple	Insectary	275	Yes	Yes	July 24	Sept. 13	17
21	Hawthorn	Apple	Apple	Apple trees	77	Yes	Yes	July 24	Sept. 13	9
22	Hawthorn	Blueberry	Blueberry	Collecting cages	114	Yes	Yes	July 25	Sept. 13	0
23	Hawthorn	Hawthorn	Blueberry	Insectary and bushes	70	Yes	Yes	July 26	Sept. 13	0
24	Hawthorn	Apple	Apple	Insectary	160	Yes	Yes	July 29	Sept. 13	59
25	Hawthorn	Hawthorn	Apple	Insectary and apple trees	63	Yes	Yes	Aug. 1	Sept. 13	36
26	Hawthorn	Apple	Apple	Insectary	275	Yes	Yes	Aug 2	Sept. 13	35
27	Hawthorn	Hawthorn	Hawthorn	Insectary	180	Yes	Yes	Aug. 7	Sept. 13	73
14	Pear	Apple	Apple	Apple trees	55	—	Yes	Aug. 1	Sept. 13	3
18	Pear	Apple	Apple	Insectary	166	—	Yes	Aug. 1	Sept. 13	0
32	Pear	Apple	Apple	Insectary	140	Yes	Yes	Aug. 5	Sept. 13	0
19	Plum	Apple	Apple	Insectary	235	—	(?)	Aug. 1	Sept. 13	0
30	Plum	Apple	Apple	Apple trees	32	—	No	Aug. 1	Sept. 13	0
34	Chokecherry	Apple	Apple	Insectary	140	—	—	Aug. 7	Aug. 30	0

In Cage 5 it will be noted that in the cross between apple females and blueberry males, positive results were obtained and 26 pupae were collected, which further confirms the work of the previous year.

Cage 12 is probably the most interesting of all. Here blueberry males and females collected from blueberry bushes produced 14 pupae in apples. So far as the writer can determine this is the first record of the blueberry form breeding in apple.

In Cage 15 a cross between apple females and hawthorn males on apple gave only one larva, which was found dead in an apple. In Cage 33 hawthorn flies which emerged in the insectary deposited eggs in apples, and 17 larvae developed and pupated.

Apple males and females appeared to behave in a normal way on the hawthorn, and pupae were procured from cages that contained flies that emerged in the insectary, as well as from those collected from apple leaves (Cages 20, 21, 24 and 26). Also, hawthorn females mated with apple males in Cage 25 produced offspring, and 36 pupae were procured.

The blueberry males and females on hawthorn in Cage 22 failed to produce pupae, although evidence of stinging was quite distinguishable and the flies appeared to be quite contented and lived a considerable length of time. An interesting observation made in connection with these flies was that they appeared to have difficulty in penetrating the haws to deposit eggs, and it may be that for this reason no maggots or pupae were found. A similar observation was made in connection with the blueberry flies on apple, although in one cage they did manage to deposit eggs. In Cage 23, containing hawthorn females and blueberry males, negative results were obtained.

Apple males and females were placed in cages on pear, plum and chokecherry, *Prunus virginiana* L. In the last two, negative results were obtained, although the plum is recorded as a host by a number of authors, and the writer has taken larvae from an unknown variety of very early soft plum in Nova Scotia. In one of the cages on pear (No. 14), where flies collected from apple leaves were used, three pupae were obtained. This confirms the observations of Porter (29) and others that larvae of this species are occasionally found in pears.

One interesting observation made in these studies was in connection with the relative activity of the flies on the various hosts. Woods (35), Patch and Woods (26) and Caesar and Ross (6) point out that apple flies are much more sluggish than those found on the blueberry. This is true to a certain extent, but then again the hawthorn flies are more active than the apple flies, and the writer found them more difficult to collect than blueberry flies. It was noted that apple and hawthorn flies could be observed and captured much more easily on a bright, calm day, but on such a day it was difficult to observe blueberry flies, and it is thought that the direct rays of the sun, especially in the middle of the day, are too hot for them and they keep in the shade. They may be observed and captured more easily between eight and ten o'clock in the morning and from four to six o'clock in the afternoon. The ideal kind of day to make observations on these flies, or to collect them, is a calm, dull, sultry day; and it was observed on more than one occasion that they could be taken with more than usual ease if an electrical storm was approaching.

It was found to be no more difficult to study blueberry flies in the field, when one had learned their habits, than apple flies. When one is looking down into a mass of vegetation it is more difficult to make observations, as the contrasts are much less sharp, than when examining leaves which are somewhat removed from other vegetation, as in the apple.

Discussion of Results and Conclusions

It is difficult to draw definite conclusions from the data obtained, since some of them are conflicting and not entirely clear cut. However, as pointed out by Porter (29), negative results in breeding work are of no significance; since none of these flies were reared successfully in captivity until Fluke and Allen (14) worked out a satisfactory feeding mixture. The writer did not get positive results in crossing from one host to another until milk was added to the diet, and one cannot predict what may happen with further work on diet and improved methods of handling and breeding.

The writer has made many field observations regarding the habits of these flies, that are not enumerated above, but which have been reported on by other authors. When one observes hawthorns absolutely free from maggots year after year growing within a few feet of apples or blueberries that are very heavily infested, and *vice versa,* it is easy to see how the conclusion may be reached that there are some fundamental differences among these flies. However, since it has been possible in some cases, by using improved methods of feeding, to induce them to adopt hosts other than those on which they were reared, may it not be possible to obtain even more striking results if the proper environmental and dietary conditions are found?

Rhagoletis pomonella (Walsh) has generally been considered as an insect with food habits approaching the monophagous condition, and various authors have referred to it as having "strains" or "forms" feeding on and developing in certain specific fruits.

Some writers, particularly Curran (9–11), regard the forms feeding in the blueberry and the snowberry as distinct species. After spending considerable time studying flies and larvae from the different fruits, the writer is of the opinion that only one distinct species is involved, and that there is not sufficient evidence on the basis of biological or morphological interpretations, or both, to prove the existence of more than a single species. It would appear that a reasonable interpretation would be to regard *Rhagoletis pomonella* (Walsh) as an oligophagous insect, in which the "host selection principle", as enunciated by Hopkins (19), has become highly developed. This theory is quoted from Hopkins (19) as follows: "That an insect species which breeds in two or more hosts will prefer to continue to breed in the host to which it has become adapted." One of the first entomologists to record observations in this connection was Walsh (33) who described the species now under consideration. However, numerous experiments which indicate that this phenomenon is not of rare occurrence in insects have been reported. Craighead (7) carried on investigations for six years with certain cerambycid beetles in the United States. Two of his conclusions that appear to have a bearing on the point under discussion are quoted here:

(i) "In general, the fewer the hosts in nature, the more marked the predilection for a particular host, and *vice versa*;"

(ii) "Continued breeding in a given host intensified the preference for that host."

The work of Glendenning (16) in British Columbia on the satin moth, the observations by Fryer (15) in connection with apple capsids, the work of Thorpe (32) on *Hyponomeuta padella* L. in England, and of Pictet (28) in France, and of others, lend weight to this theory. Until such time as further investigations have disproved this hypothesis, it would appear to the writer a reasonable one to accept.

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CONCEPTS AND MECHANISMS OF RESISTANCE IN HELMINTHIC INFECTIONS[1]

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Abstract

The relations that exist between the helminths and their hosts cannot be divided into specific groups. Nevertheless, it is desirable that some classification of these relations, together with clear-cut definition of terms, be widely accepted in order that the subject may be discussed intelligibly. A tentative classification is suggested, which distinguishes between (i) compatibility and incompatibility, which refer only to the host environment as it exists before invasion by the parasite; (ii) resistance, of various kinds and degrees, which refers to the reaction of the environment to the *presence* of the helminth; and (iii) tolerance and intolerance, which refer to the reaction of the environment to the *effects* of the helminth.

The science of immunology deals with the reactions of the body to foreign irritants, organic and inorganic; the organic irritants may include both animal and vegetable parasites, as well as their own products and the products of non-parasitic organisms. Until recently, however, the science has largely been studied as a branch of bacteriology or, to a lesser extent, as a branch of protozoology. Within the past few years, the effects of helminth parasites on the host and the response of the host to these parasites have been under investigation in many laboratories, and numerous papers have been written on the subject. The terminology used, however, has been the terminology of the bacteriologist and, in a number of cases at least, this has resulted in some confusion.

The metazoan parasites are very different in their mode of life from the protista and it does not seem to be possible to interpret their effects without reference to their own actions. The host, on the other hand, is the same, and all the evidence points to an essentially identical mechanism of defense in the host. There is a definite need for a terminology which, describing accurately the relation between host and helminth parasite, will avoid confusion with the already established concepts of the bacteriologist and the protozoologist, and yet will enable the pathologist to reconcile all three points of view.

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The word "helminth," although it has a certain practical utility, is undoubtedly a bad one to use in scientific terminology, and although every definition lays emphasis on the fact that it is a faunistic term, this has seldom been properly appreciated in discussions on immunity. It is necessary to remember that the helminths consist of several completely unrelated phyla, or parts of phyla, which have become adapted through the process of evolution to live in a parasitic environment. To do this, they have adopted many different devices—and their different modes of life are just as varied as those which exist among the vertebrates which they mainly parasitize. It is accordingly very dangerous to draw conclusions of a general nature from observations on a single species. This is especially so when it is realized that we know practically nothing about the physiological processes of the various species. Yet it would seem to be obvious that a helminth that has a purely blood diet, and one that feeds on tissue, bile, mucus or other substances, require different sets of environmental conditions.

The helminth parasite is quite different from the protozoan or bacterial parasite. It enters the host terrain as a young form, grows to adult life, passing as it does so through several stages, and often undertaking an extensive migration through the host tissues; finally it settles down to adult life, mates, and produces eggs or larvae, all of which must ultimately leave the host before they can continue their development. Outside of the host they undergo a certain number of larval changes before they are infective to a new host.

No known helminth can complete its life cycle in the same individual; sooner or later the eggs or larvae must leave the host, and if they are to survive, sooner or later they must gain access to the same or another host. (Even in such apparent exceptions as *Hymenolepis nana*, the eggs normally leave the intestine in the feces to return by the mouth and pass their larval stages in the villi of the small intestine.) This is the fundamental difference between helminths and other parasites.

Helminth parasites must be considered as a collection of individual units each entering the host separately. Bacterial or protozoal parasites are quite different. One enters the body and there it multiplies—sexually or asexually—until an infinitely large number results. They are at best simple creatures, without the complicated morphology, and consequently complicated growth to maturity, found in the metazoan parasites.

The parasitic life of a helminth is dependent on its finding a suitable environment in which it can develop to maturity and in which it can reproduce successfully. This postulates a number of favorable factors.

Factors in Successful Reproduction of Helminths

(a) Successful Admission to the Host

The host must traverse the ground where the free stages of the parasite are found; it must eat suitable foodstuffs in or on which the larval stages occur; it must be exposed to the intermediate host, and so on. Even slight differences in habits, *e.g.*, such as exist between a sheep and an ox in eating grass, may make all the difference in the parasite gaining admission. The anatomy of the host, including the thickness of the skin, are factors to be considered under this heading.

(b) Suitable Environmental Conditions in the Host

Once inside the host, the parasite must find a suitable habitat—type of mucosa in the intestine, length of intestine, presence of suitable food, and so on. There is a belief, which is not so strongly held now as it once was, that the temperature of the host is of paramount importance, especially in warm-blooded animals. The temperature in edentates however is by no means constant, and yet no wild animal that I have examined has a larger burden of parasitic worms—including forms found in species with more or less constant temperatures—than has the lesser anteater of South America. The influence of hibernation is not well understood nor is the influence of fever. However, clinical experience has shown repeatedly that a high fever will often cause a large proportion of helminths to leave the body, just as an enteritis will cause many intestinal forms to pass out.

(c) Possession of a Suitable Protective Mechanism Against the Normal Metabolic Processes of the Host

When the parasite lies in the alimentary system, it must possess some means of preventing itself being digested or being passed out by peristalsis, etc.

(d) Absence of a Host Reaction that would Interfere with the Normal Metabolism of the Parasite

This factor applies most obviously to parasites that leave the lumen of the alimentary tract at some period of their life, but it may apply to all helminths. If there is any host reaction, the parasites must be able to resist its effects.

These groups of factors are essential for the proper development of the parasite to full and functional maturity. If it finds the environment suitable for its development, we may define the condition as "compatibility"; if it is unsuitable, it is "incompatibility". If the parasite dies at once or is passed out unchanged, the condition is "complete incompatibility". If it develops normally with no unfavorable results to either itself or the host, the condition is "complete compatibility". However, intermediate stages exist and the parasite may grow to some intermediate extent. This intermediate extent may stop short of full maturity; the parasite may never be able to reproduce. On the other hand, it may become mature but not fully so; the adults may be small, the egg output reduced and so on. To enable these to be distinguished from each other, the first is called "partial incompatibility", the second "partial compatibility". None of these conditions has any connection with a host reaction; they merely define the type of environment that the helminth finds in the host.

In complete incompatibility and complete compatibility, no host reaction can occur, but in both partial incompatibility and partial compatibility, a reaction may or may not be present, and this reaction may be directed towards the *effects* of the parasite or towards the *presence* of the parasite.

The reaction of the host to the presence of the parasite is termed "resistance". When the resistance is sufficiently high to prevent the parasite from reproducing (*i.e.*, producing eggs or larvae) it is termed "absolute resistance". This may act at any part of the parasite's life, prior to the actual production of eggs or larvae and it is possible to conceive of it being so pronounced as to destroy the parasite immediately on entry. This theoretical conception, which may be referred to as "virtual resistance", has the same result as complete incompatibility, but differs from it in that it assumes a reaction on the part of the host; complete incompatibility on the other hand, assumes an unsuitability of environment, not necessarily inimicable to the parasite —it may be the absence of something. When the parasite is able to reproduce, in spite of the host reaction, the resistance is termed "partial resistance"; the life of the parasite must in consequence be, to some extent at least, abnormal.

The reaction towards the effect of the parasite is essentially a repair process, whereas resistance involves an active alteration in the parasite's environment. In our present state of knowledge, it seems desirable to keep these two concepts separate. Where the damage done is of a purely mechanical nature for example, it is possible that the reaction is directed entirely towards the repair of this damage and does not affect the worm itself. If the reaction towards the effects is sufficient to counteract and repair them, it is referred to as "tolerance"; if not, then it is called "intolerance". These terms are comparative and depend on a great number of varying factors and they must grade into each other to some extent; intolerance postulates clinical disease—tolerance, sub-clinical disease with no obvious symptoms.

Compatibility, incompatibility and resistance may all be natural or acquired, but an intermediate form of acquired resistance may exist called "premunition." In this form some parasites may develop—either successfully or unsuccessfully—but so long as they remain alive, resistance is offered to further infection; as soon as they die or pass out of the host, the resistance will disappear.

These terms have been defined as though they were hard and fast divisions; it is improbable that they are. Thus too many worms in a completely compatible host would induce a state of incompatibility through lack of food. Tolerance and intolerance obviously grade into each other and both absolute and partial resistance can exist side by side in the same host towards the same species of worm. Premunition may precede and grade into acquired resistance. Moreover, it is at least doubtful whether complete compatibility, incompatibility or resistance are more than theoretical concepts. However, any particular state is true absolutely, not comparatively, for any individual worm.

This scheme accordingly proposes the recognition of five concepts in the relation between host and helminth parasite.

Compatibility, as it refers to the environment as it exists before the invasion by the parasite, will be discussed first. Resistance, as it refers to the reaction of the environment to the presence of the helminth, will be discussed next, and tolerance, as it refers to the reaction of the environment to the effects of the helminth, will be discussed last.

Compatibility

"Compatibility" expresses the idea that the host environment is suitable for the successful development of the parasite, "incompatibility", that the environment is unsuitable. Compatibility does not imply any relationship with virulence as does the term susceptibility in bacteriology; this follows from the fundamentally different life history. In a susceptible host, bacteria will multiply indefinitely; in a compatible host, the number of worms will depend on the number of infective stages gaining admission. There is no evidence at all that the term virulence can be used in connection with helminths in the sense in which it is used in bacteriology.

Obviously, compatibility and incompatibility can be complete or partial. "Complete compatibility" expresses the theoretical idea that the host environment is completely suitable for the development of the parasite. It postulates the absence of any reaction or resistance to the worm as well as the presence of all the necessary environmental factors and it is exactly equivalent to a state of commensalism. Obviously, however, no host can be infinitely compatible as there must be a mechanical limit to the number of parasites harbored, and so this state must grade into incompatibility. Too many parasites may cause a stunting in growth or a reduction in egg output, merely by their own number and without causing any form of reaction in the host.

"Complete incompatibility" expresses the theoretical idea that the host is completely unsuitable for the development of the parasite. It also postulates no host reaction, as the environmental conditions are unsuitable in nature before the advent of the parasite. It is a form of passive protection, but as the word "passive" is used in bacteriology with an entirely different meaning, it seems inadvisable to use it here.

There must exist, however, several intermediate conditions of environment between complete compatibility and complete incompatibility.

The parasite may grow to maturity and lay eggs, but it may be abnormally small in size, or produce abnormally few eggs or live a shorter time than normal. This is defined as "partial compatibility", and expresses the idea that the host environment is more or less suitable. In this group of conditions would be included all cases where there is a reaction on the part of the host to the presence of the mature parasite, as such a reaction must cause some abnormality, no matter how small, in the life of the helminth. Partial compatibility, however, has no necessary connection with any host reaction.

If the parasite grows to some extent, but does not reach maturity, the condition is one of partial incompatibility. The parasite dies or is passed out because conditions are unsuitable, but not so unsuitable as to cause it to die on entry or to be immediately passed out. This state may or may not be associated with any form of host reaction to the presence of the parasite; it has, however, no necessary connection with any form of resistance.

It is quite possible that both complete states are purely theoretical concepts that do not exist in nature and that the partial states are the only ones which occur.

It is probable also that compatibility or incompatibility may be natural or may be acquired by changes in diet, concomitant disease and so on; the so-called "age resistance" may, for example be an acquired incompatibility in mature life and a natural compatibility in youth. The presence or absence of vitamins, minerals and other dietary factors appears in some cases to have much to do with rendering the environment suitable or unsuitable, although as our knowledge advances we may find that these factors are concerned mainly with resistance.

Resistance

If worms fail to become established in a host, it must be because the environment is unsuitable for their establishment, assuming that the larval stages are normal and healthy and can gain admission. This may be the result of incompatibility, which postulates the absence of something essential for the worm's well-being, or resistance to their presence, which postulates the presence of something produced by the host, which is inimical to the parasites. This something may be normally present in the host or it may be produced only on the introduction of the parasite or after some equivalent stimulus; it seems impossible in the present state of knowledge to differentiate sharply between incompatibility and resistance, and it seems difficult to draw a line between natural and acquired resistance. However, such differences must exist.

It is essential, moreover, to select some criterion of resistance against helminths, as "protection" may be shown by the immediate death of the worm or its death at any subsequent period or by its physiological processes being altered so that its life is shortened, its growth stunted, its egg output reduced or its habitat abnormally situated. On the other hand, the parasite may pass out of the body in an active larval stage capable of developing in another animal, or may pass into a quiescent larval stage which also can develop in another animal.

The picture is still further complicated by the fact that the helminths are active animals and may move away from any unfavorable environmental influence, just as can any other animal. It is difficult, therefore, to say that a larva passed in the faeces after entry to the host, has been subjected to any different harmful stimulus from one which was killed *in situ*.

The most perfect form of resistance against any worm would cause its immediate destruction upon entry. Although this might be called "immunity", that term is already used in bacteriology with a different meaning, and until more is known of the parasitic life of helminths and the body response to them, to avoid confusion, the term "virtual resistance" (which is not the same as absolute resistance) is suggested as preferable. This is especially so because complete incompatibility would have an apparently identical result so far as the result is concerned, yet would involve no special body reaction. However, if as the result of a host reaction to its presence, a certain species of worm in a host cannot produce eggs or larvae capable of continuing the cycle, it would be perfect resistance from the biological point of view and this would seem to be our logical concept of absolute resistance.

In practice, while virtual resistance has yet to be proved, it appears that an absolute resistance can and does exist in some cases, although no host is absolutely resistant to all species of helminths. Many of the examples of absolute resistance however, are undoubtedly attributable to external causes, such as climate, opportunity and habits due to race, age, sex, etc.; the animal simply is not exposed to infection. When it is exposed to infection, sooner or later, it acquires a great variety of helminths. Man himself is the best example, because his parasites have been most extensively studied. He has few that are specific to himself alone. Of these, but one genus *(Enterobius)* is shared with other primates and can be considered as a natural species. Practically all the others that occur in human beings are either identical with, or closely related to, forms found in domestic or other animals. It is difficult to escape the conclusion that exposure to infection from habits—especially food habits and close association—is the main factor that decides whether a given parasite occurs in a given host. We have been badly handicapped in our knowledge of this subject by the fact that study has tended to concentrate on the parasites of man and those animals closely associated with them.

As our range of experience extends we are finding that host-parasite specificity is much less common than is generally supposed. Our present information is that it appears highest among the cestodes—but we know even less of their biology than of that of any other worms. It appears lowest among the trematodes. To mention only a few of many possible examples, the common liver fluke *(Fasciola hepatica)* occurs in practically every mammal from man to marsupial; Swales (6, 7) and Krull (4) have recently found that the related *Fascioloides magna* will parasitize a wide variety of hosts; *Brachylaemus* of the opossum will live in dogs, cats and young chickens; the Opisthorchidae and the Heterophyidae have an even wider range of hosts; *Sticorchis* of peccaries will not only live in pigs, but also in edentates and rodents.

Among the nematodes, forms with intermediate hosts tend to show less host specificity than those with a direct life history. Heavy infestations of *Spirocerca* of carnivores, for example, have been found in such animals as donkeys which have been given the opportunity of feeding on dung beetles

(5), while *Dracunculus* occurs in a wide variety of hosts in various parts of the world. Many different species of nematodes with a direct life cycle have been developed experimentally in other animals; thus Daubney in East Africa has recently reported the increasing success with which nematodes of wild ruminants are becoming established in sheep, while the genera *Trichostrongylus* and *Strongyloides*, among others, have a wide variety of natural hosts.

Moreover, many helminths that have difficulty in developing in a "normal" host, can reach maturity after host dietetic modifications, such as avitaminosis, disturbance of mineral balance, starvation, and so on.

While the data are scattered and very incomplete it would seem that physiological similarity is much more important than blood relationship in connection with helminth resistance. Helminths are subject to the same evolutionary laws as are other animals, and sooner or later a mutant arrives which is capable of living in the new environment. The closer the physiological similarity of the new to the old host, the greater will be the opportunity for this.

It follows, accordingly, that a state of partial resistance must exist in many cases, in which the host reaction, while not sufficiently severe to prevent the worm from reaching sexual maturity, yet prevents it from reaching its full size, shortens its life, or only permits a small number of the larvae which gain admission, to develop. A similar result may, of course, follow partial compatibility, and it is not always possible to differentiate those two states, *e.g.*, *Clonorchis* is abnormally small in cases when a large number is present—probably partial resistance—or when in an unusual host such as a rabbit—probably partial compatibility. A similar effect is observed in dogs with a heavy ancylostome infection, when the egg output per worm is considerably reduced.

NATURAL AND ACQUIRED RESISTANCE

Natural resistance is resistance to original infection and is the result of an inherited constitutional peculiarity of the host which interferes with the normal development of the parasite. It is usually a species resistance and cannot usually, in the present state of our knowledge, be separated from incompatibility. It may be absolute or partial.

Natural resistance does not always prevent the entrance of the parasite. Cercarial dermatitis is an example of the failure of the human body to prevent the entry of the larval stages of schistosomes that normally develop in other animals and never develop to maturity in man; so far as our present evidence goes, the human body appears to be naturally absolutely resistant to these worms. In the case of the human "creeping disease" due to dog hookworms, a similar state of affairs exists, except in the case of *A. braziliense* where the natural resistance is partial instead of absolute.

It follows from these examples that, while much natural "resistance" is obviously incompatibility, some is undoubtedly active, especially in helminths, the parasitic life of which is not restricted to the lumen of the alimentary canal. The fact that an active natural resistance can exist, means that the host response must differ in different species of animals.

"Breed" resistance or incompatibility is closely related to species resistance or incompatibility. Webster has shown that a breed resistance against bacteria exists in mice (as well as a breed susceptibility) and that it can be enhanced by inbreeding. This resistance is to some extent non-specific, as in addition to mouse typhoid, the response against corrosive sublimate was increased. Ackert *et al.* (1) have found some evidence of a breed resistance against Ascaridia in chickens and there is some evidence of a breed susceptibility to *Haemonchus* in sheep.

"Age" resistance is generally taken to mean a "resistance" which increases with the age of the host in the complete absence of infection. There is a considerable amount of evidence to link it with incompatibility, although some of the examples are really acquired resistance. It is generally shown towards parasites of fairly closely related hosts. The young animal has a more generalized type of physiology than the adult and there is a fundamental similarity between the milk-fed young of all mammals; later, when they assume the feeding habits of the adult, the similarity becomes less marked. Moreover, young animals experimentally eat objects that adults refuse and their skin is more easily penetrated by larvae. Accordingly, they should, on first principles, have a larger range of parasites than have the adults of the same species. However, some workers believe that some factor (or factors) is lacking in the young animal, which develops in the adult as the natural consequence of age, in the complete absence of infection (3).

It is impossible at present to differentiate sharply natural resistance from acquired resistance. A resistance evoked against one species may operate to some extent against another. Natural infections—especially those which have not progressed beyond the larval stages—are difficult to eliminate in practice.

Acquired resistance follows and is due to previous infection with helminths; it may be absolute, but is usually partial. Such evidence as is available suggests that the intensity of such a resistance is more or less directly proportional to the amount of tissue damage. Thus, in sheep (8), resistance to *Haemonchus* and *Nematodirus*—blood-sucking nematodes—is more quickly produced and more active than resistance to *Ostertagia*, *Trichostrongylus* and *Cooperia*—non-blood-sucking nematodes. Resistance to larval cestodes developing in somatic positions is relatively great. The few cases of artificially acquired resistance of which we have any knowledge are in this last group. Some workers have also found that they can induce a partial resistance in dogs against *Echinococcus* adults, by the injection of larval cystic material.

Premunition is related to acquired resistance but exists only so long as an original infection with the same species is present; true acquired resistance is subsequent to the infection and survives it. However, premunition may precede true acquired resistance, or may exist simultaneously with the building up of acquired resistance. It is possible that at least some cases of premunition are due to the accumulation of products of the parasites which make the environment unfavorable to new parasites; such an action might be quite independent of any host reaction and should be classed as acquired partial incompatibility. Undoubtedly many of the examples of the so-called premunition against helminths are based on insufficient data. Some, such as *Hymenolepis nana*, are probably not premunition but true acquired resistance, the cysticercoid stage in the villi having caused the resistance, rather than the adults which have developed from them and are still living in the intestine. Premunition, if it applies at all to infections with helminth parasites, must apply to an infection with more than one individual. The original individual, which induces the resistance, must be able to survive a reaction which forms that enter the host subsequently are unable to survive.

Mechanism of Resistance

Although the mechanisms of active resistance against helminths have been little studied, there is no reason to believe that they differ in essentials from those brought into play against any other foreign invaders. Obviously they must vary from helminth to helminth, depending upon the life processes of the particular parasite under consideration. It is probably true that connective tissue cells form the main body defense and that the response is mainly local rather than general. There is no evidence that any worm produces a toxin in the bacteriological sense, and such noxious substances that do enter the body seem to belong to the class of "foreign proteins," and are the result of the normal metabolism of the parasite.

The presence of eosinophile leucocytes in the blood and other tissues is very common in helminthic infections. There is an increasing amount of evidence to suggest that the function of the eosin-staining granules in these cells is to provide an antidote to foreign proteins, although the cells themselves have phagocytic properties, engulfing and digesting organisms and cells; this property appears to be secondary, however, to their property of rendering foreign proteins innocuous. They are accordingly common in anaphylactic phenomena. A local eosinophilia can be produced only in animals showing eosinophiles in the blood. When these are absent, the eosinotactic substances provoke a considerable accumulation of neutral polynuclear cells. Once this local eosinophilia is established, however, it may persist after the disappearance of the general eosinophilia, and during its establishment it may prevent the appearance of a blood eosinophilia by withdrawing the eosinophiles from circulation.

The general body responses against these dissolved substances are apparently quite strictly analogous to those produced in bacterial infections and there is no need to postulate any special mechanism. As in the case of bacteria, it appears to be affected by defects in diet (including avitaminosis). Ledingham points out that while there is little evidence that nutritional defects disturb the antibody-forming mechanism of the body, there is ample proof that resistance both to spontaneous and induced infection, is profoundly affected. The precise parts played by particular vitamin deficiencies in producing this lowered resistance are not yet clear. Generally restricted diets may act similarly to diets lacking particular vitamins. The normal excitability of the reticulo-endothelial tissue is greatly reduced by defects of diet which lead to loss of weight. There is a considerable amount of evidence to show that this applies equally to helminths as it does to other foreign stimuli.

Hypersensitivity as the result of helminth infections is a relatively common phenomenon, and allergy is probably a protective mechanism—although it may act harmfully—and it is possible that at least some of the symptoms produced by helminths are the result of hypersensitivity to a relatively harmless parasitic protein. Its normal function is possibly to stimulate a rapid attack on the invading organism or substance and either immobilize or destroy it.

The outstanding reaction against worms however is a local fibrous proliferation. It is the usual inflammatory response which follows the local introduction of any foreign stimulant into the connective tissue of any normal host and serves to isolate the parasite and ultimately to destroy it and repair the tissue damage. Whether the destruction is active or passive is still unknown.

While this reaction is similar in all species of animals it varies considerably in detail from host to host and there is a very urgent need for more detailed studies in comparative pathology. By this is meant a study of the reaction of a variety of hosts to a single cause—such as has recently been carried out by Swales with *Fascioloides magna.* Although known as the "giant American cattle fluke," it is not a normal parasite of cattle. It lives normally in the liver substance of various members of the deer family, especially in elk (or wapiti), coast deer, mule deer and Virginia deer. The lesion in the liver of these animals allows the free exit of ova and thus the life cycle is completed in the presence of suitable snails. However, large Bovidae, including cattle, ranging over the same territory as the deer, ingest the infective cysts on grass, etc., and become infested, the liver appearing dark and irregular in outline and individual lesions appearing, similar in external appearance to common abscesses. The tissue reaction to the presence of the flukes in these animals results in a high eosinophilia and the formation of a hard fibrous cyst from which all means of exit for the eggs are occluded. Thus the parasite is completely enclosed even before it grows to maturity, and its destructive migrations and means of reproduction are stopped. Cattle, accordingly, are absolutely resistant to the presence of this parasite. Sheep, on the other hand, are unable to resist the fluke's migration in the liver tissue and the parasite is, therefore, extremely pathogenic to them and, at least in some cases, death of the host appears to precede the maturation of the parasite. Infected sheep livers appear as obviously diseased organs; discoloration, irregularity of outline and fatty infiltration predominating in the picture. Sheep, like deer, are partially resistant to the presence of the fluke, although their reactions to its effects are different.

Tolerance

Only the effects of the parasite on the host are concerned with disease, and these effects may be due to a variety of causes, negative as well as positive. Apart from the abstraction of foodstuffs and the destruction of other organisms that may be of value to the host, helminths may cause traumatic and other lesions, or produce soluble substances in their excretions or secretions, which are harmful to the host; there is no evidence that any produce toxins in the bacteriological sense. There must be a host reaction to these effects, and if this reaction is sufficient to counteract the effects of the parasite, a state of tolerance may be said to exist; if it does not, then the host is intolerant to the effects of the parasite. It implies no necessary resistance to the *presence* of the parasite although the mechanism of tolerance may be identical with that of resistance. On the other hand it may be quite different. Thus, resistance to the effects of blood-sucking nematodes (such as *Haemonchus*) may be shown by unusual activity on the part of the blood-producing organs, while resistance to their presence may be produced by the connective tissue system. Tolerance will depend upon many factors, external as well as internal, and will vary with individuals, age, diet, intercurrent disease, and so on, and will range from complete tolerance to complete intolerance through an infinite variety of stages. Tolerance can probably be best considered as a repair process and there seems to be no necessity for postulating any specific mechanism different from that used to repair the damage to the host by any other agent. When this process involves a reaction to a dissolved agent, there may be alterations in the serum of the host detectable by test antigens. These do not necessarily play any part in the resistance of the body to the presence of the parasite, any more than do local repair processes necessarily play any part in this resistance.

Tolerance thus used is a comparative term and seems only capable of definition if we assume that it represents the maximum position, *i.e.*, that the body defense against the effects of the parasite is strained to its utmost and that any further increase in effect would break down tolerance. However, any lessening of the effect would not affect the reaction and so tolerance must include all stages from those of an infinitely small nature to those which create the maximum reaction. Beyond this theoretical limit—which must be susceptible of infinite variation due to other causes—we have intolerance, and with intolerance we have the beginning of disease. The greater the intolerance the greater the disease, until at its maximum the host dies. Tolerance on the other hand means the absence of any clinical disease and it may even mean the complete absence of any host reaction to the effects of the parasite because the parasite may have absolutely no effect at all on the host. It expresses the balance of the host-parasite association.

Owing to the delicate adjustment between a host and the species of parasite which is normally found in it, there is less probability of disease than when either host or parasite is unusual. During the process of evolution, the ecology of such a parasite has become so adapted that it does the minimum amount of damage to the host and so to the chances of continuance of its own species. This adaptation includes not only physiological adaptations of the worm to the parasitic existence in the body, but adaptations of the external larval stages, so that, under normal circumstances, sufficiently few gain admission as not to endanger the host, but not so few as to endanger its own species survival. It is only when these conditions are upset that clinical disease results.

When the parasite is in an unusual host a relatively small number of helminths may cause quite extensive reactions.

Accordingly, intolerance, accompanied by clinical disease is due, as a rule, to large numbers of "usual" parasites or to small numbers of "unusual" parasites in a host. Thus the case of *Fascioloides magna* mentioned above; sheep are very intolerant while the deer tribe are very tolerant to the effects of the parasite.

Relation Between Intermediate Host and Parasite

Many helminths pass part of their larval existence in various animals, called intermediate hosts. In these animals, the parasite cannot (or at least usually does not) reach sexual maturity. There are certain exceptions to this however. In certain species of trematodes for example, eggs may be found in the "cercarial" stage in snails, although there is no evidence that they are normally deposited during this stage. In some cestodes, such as *Taenia solium* in man, the larva may develop to an infective stage in man, as well as in the normal intermediate host, the pig; in this case, however, they cannot develop further and remain as larvae, unless of course, they are eaten by another man.

The host-parasite relation between a larval parasite and an intermediate host would seem to be strictly analogous to that existing between an adult parasite and a definitive host, although the criteria adopted must differ. If for "sexual maturity," the expression "infective stage," is substituted, the concepts of Compatibility-Incompatibility, Resistance, and Tolerance-Intolerance would seem to apply to them also.

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THE PARASITES OF PIGEONS IN CANADA[1]

By M. J. Miller[2]

Abstract

All the helminths found in the domestic pigeon are reviewed and notes on their distribution, location in the host, and pathogenicity are given. Three helminths, *Ascaridia columbae*, *Capillaria columbae* and *Echinostoma paraulum*, have been found in Canadian pigeons and described, *Echinostoma paraulum* being recorded for the first time in North America. The lip characteristics of *Ascaridia columbae*, which until now have been little studied, are described in detail.

The internal parasites of pigeons have been known almost as long as the study of systematic helminthology itself. As far as can be gathered from the available literature, the first mention of a parasite from the domestic pigeon was in 1782 when Goeze recorded *Ascaris teres*. Since then a considerable number of helminths has been described, the three major classes being well represented with members of several families in each class.

A complete list of the helminths that parasitize the pigeon is given at the end of this paper. In the case of those parasites found in other domestic birds as well as in pigeons, the distribution as found in all hosts is given. A few species from the pigeon recorded by earlier authors, and not referred to in the later literature, are indicated by a question mark.

Trematoda

Location in Host

As far as is known all the trematodes of the pigeon are found in the small intestine. The exact position is not constant but usually includes the duodenum and the anterior part of the ileum; however, *Brachylaemus commutatus*, being a caecal dweller in other birds, would most likely be found in the posterior part of the small intestine.

Pathogenicity

By far the most harmful of the trematode parasites in pigeons is *Echinostoma paraulum*. Mönnig (20) states, "Krause and Van Heelsbergen in Europe and Picard in Java have observed deaths in pigeons caused by this parasite. The birds show inappetence, thirst, diarrhoea, lassitude and progressive weakness. At post mortem there is a slight atrophy of the breast muscles and catarrhal enteritis with much mucus, becoming haemorrhagic behind the duodenum."

Echinoparyphium recurvatum causes emaciation and anemia in fowls and would probably produce similar symptoms in pigeons. According to Neveu-Lemaire (24), the presence of *Brachylaemus commutatus* in certain cases causes a hemorrhagic typhlitis. *Hypoderaeum conoideum* is responsible for localized enteritis in ducks and would probably produce the same condition in pigeons. *Apatemon gracilis* has not been reported as being harmful.

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Cestoda

Location in Host

All the tapeworms of pigeons occur in the small intestine. According to Meggitt (19), they are found mostly in the duodenum, *Hymenolepis* nearer the stomach, *Cotugnia* and *Raillietina* farther away. He concludes however that under normal conditions the worms wander from place to place.

Pathogenicity

Generally speaking the cestodes are less harmful to their host than are the other classes of helminths. However, when they become very numerous there is a pronounced effect on health. Meggitt (19), who has carried out considerable work on cestodes of pigeons in Rangoon, writes that extreme emaciation and weakness were the usual accompaniments of a heavy infection. In young birds a heavy infection would probably be fatal.

Nematoda

Location in Host

The nematodes have a wider range in their adaptation to different locations in the host than have the trematodes and cestodes, and they are found from the crop to the posterior part of the alimentary tract. The spirurid worms are usually found in the proventriculus, but may be found in the oesophagus and crop as well. *Acuaria spiralis* is also found in the small intestine. *Ascaridia columbae* is found in the duodenum or just posterior to it. *Eulimdana clava* is found in the connective tissue around the trachea and *E. mazzantii* in the connective tissue of the neck. The remaining two species are found in the small intestine.

Pathogenicity

Of the helminth parasites of pigeons, the nematodes cause the most trouble. The commonest species is *Ascaridia columbae*. Unterberger (1868) was the first to draw attention to the pathogenicity of this parasite. According to Neumann and MacQueen (23) it causes torpidity, loss of appetite, periodic diarrhoea and finally marked wasting, particularly of the pectoral muscles. Death usually occurs at this period. At autopsy, the mucous membrane is distended in patches, more or less large and numerous, engorged with blood, softened or ulcerated and covered with thick mucus. Irwin Smith (14) reports it as being responsible for the death of squabs in New South Wales. Le Roux (17) attributes the death of pigeons in Natal to this parasite. It should be noted however that *A. columbae* is a very common parasite of pigeons, and adult birds, when lightly infected, normally show no symptoms of disease, although when as many as 400 to 500 worms are found in a single bird, as has been reported, they would probably cause grave disorders. The greatest damage is done to young squabs, and among them the highest mortality occurs.

Capillaria columbae is also very common and when present in large numbers is distinctly pathogenic. Neumann (22) quotes Pauly and Zurn as stating that it often causes an intense intestinal catarrh which leads to anemia and debility. According to Neveu-Lemaire (24) autopsy of an affected bird shows the intestinal mucosa to be grayish, tumefied and covered with striae and petechiae.

Cram (8) reports heavy infections of *Acuaria (Dispharynx) spiralis* as causing severe losses among carrier pigeons of the Signal Corps of the United States Army. Symptoms described by Whitney are droopiness and loss of weight with retention of a ravenous appetite, as well as loss of pigmentation of the iris, probably due to anemia. A post mortem showed great enlargement of the proventriculus, the inner surface being a thick necrotic mass containing large numbers of *A. spiralis*. Microscopically there appeared to be almost complete destruction of the glands and a marked infiltration of the underlying tissue.

Ornithostrongylus quadriradiatus is very often fatal to large numbers of birds. According to Cram (9) 134 worms are the minimum required to cause death. This species was described in 1904 by Stevenson who mentioned it as the cause of death of large numbers of pigeons in Washington. Komarov and Beaudette (16) reporting on three flocks, state that in one, 46 out of 50 birds died; in another, 20 out of 100; and in the third flock of 17 birds, all died because of this parasite. Le Roux (17) reports the death of pigeons in Natal from the same worm. According to Stevenson, when worms are present in large numbers, death is caused by a general disorder of the nutritive function of the host due to bacterial infection of the pierced mucosa and the loss of blood.

In *Tetrameres* spp. a great deal of damage is done by the female parasite which migrates into the wall of the proventriculus causing marked irritation and inflammation. According to Timon-David (29) who studied the histopathology of *T. fissispina* in the pigeon, there is a true digesting away of the glands by the enzyme of the parasite. Neumann and MacQueen (23), quoting Zurn, state that this parasite kills young ducks. It would probably act similarly in pigeons.

Helminths of Canadian Pigeons

In Canada, according to Swales (28), two helminths have been recorded from the domestic pigeon, *viz.*, *Ascaridia columbae* (by Wickware, 1922 and Rayner, 1932) and *Capillaria columbae* (by Rayner, 1932). Rayner's records are both from Quebec.

The bulk of the material examined in the present investigation was supplied by Messrs. Henry Gatehouse and Son, Montreal. It consisted only of the posterior part of the alimentary tract, usually including the stomach, and was received in lots of 60–80 at irregular intervals. As a result the material was starting to decompose and had to be examined rather hurriedly. The remaining pigeons were obtained locally and kept in wire cages in the Institute of Parasitology. They consisted of 22 in all, 11 Homers and 11 Tumblers.

In the Gatehouse material, 232 intestinal tracts were examined and, on the whole, surprisingly few parasites were found. Sixteen echinostomes (*Echinostoma paraulum* Dietz, 1909), 12 larval nematodes and three capillarids were found. The larval nematodes were undoubtedly ascaridias and most likely *A. columbae*. In comparing them with the larval stages of *A. galli* as recorded by Ackert (1) they approximated the second- and third-stage larvae. The third-stage larvae were 6.3 mm. long; the average length of this stage in *A. galli* is stated by Ackert to be 6.4 mm. Other characters were similar to those described by Ackert. Three distinct lips are present, showing papillae (Fig. 17). In the male there is a distinct pre-anal sucker as well as certain caudal papillae (Fig. 16), and several cells comprising the primordium of the spicules can be seen. The second-stage larvae are 3.8 mm. long; Ackert figures *A. galli* second-stage larvae as 4.2 mm. long. They have lips just beginning to form, an anal prominence, but no pre-anal sucker.

Of the pigeons kept at the Institute, eight were autopsied; all but one harbored capillarids and all but three, ascaridias. The incidence of the infection is given in Table I. Fecal examinations showed that the 11 Tumblers as well as a third of the Homers were infected with ascaridias. It must be remembered, however, that in some cases a pigeon may harbor a single male and consequently not show eggs in the faeces. In the case of capillarids, fecal examinations are not as satisfactory, as the eggs appear sporadically. However, at least three quarters of the birds were infected with capillarids..

TABLE I

PIGEON PARASITES FOUND

Date	No. of pigeons	Parasites
Nov. 26	66	16 echinostomes 2 larval nematodes
Dec. 6	8	Negative
Jan. 18	78	1 echinostome 10 larval ascaridias 3 capillarids
Mar. 2	1	3 capillarids 1 ascaridia
Mar. 2	1	1 capillarid 10 ascaridias
Mar. 3	1	1 capillarid 2 ascaridias
Mar. 3	1	Negative
Mar. 26	1	1 ascaridia 7 capillarids
Mar. 28	1	3 capillarids
Apr. 17	1	7 ascaridias 1 capillarid

The ascaridias were identified as *A. columbae* and the capillarids as *C. columbae*.

The fact that from the Gatehouse material 12 larval ascaridias were obtained, and no adults, suggests that probably a definite age resistance to *A. columbae* is shown by the pigeon, as is shown to *A. galli* by the fowl (Ackert *et al.* (3)).

The pigeons exhibit some evidence of a strain resistance to *A. columbae*. This is suggested by the higher proportion of infected birds among Tumblers than among the Homers, and the fact that one of the Tumbler pigeons yielded a larva about 4.0 mm. in length, whereas in the Homers experimental infection did not produce a larva over 48 hours (about 0.36 mm.).

DESCRIPTION OF PARASITES

Ascaridia Dujardin, 1845

Generic diagnosis (From York and Maplestone). Generally with cuticular lateral flanges: oesophagus club-shaped, but without a posterior bulb. Male: pre-anal sucker slightly prominent with a chitinous rim; caudal alae narrow; papillae relatively large; spicules equal or subequal; gubernaculum absent. Female: vulva near the middle of the body; uteri opposed. Oviparous eggs with a thick shell.

A. columbae Gmelin, 1789

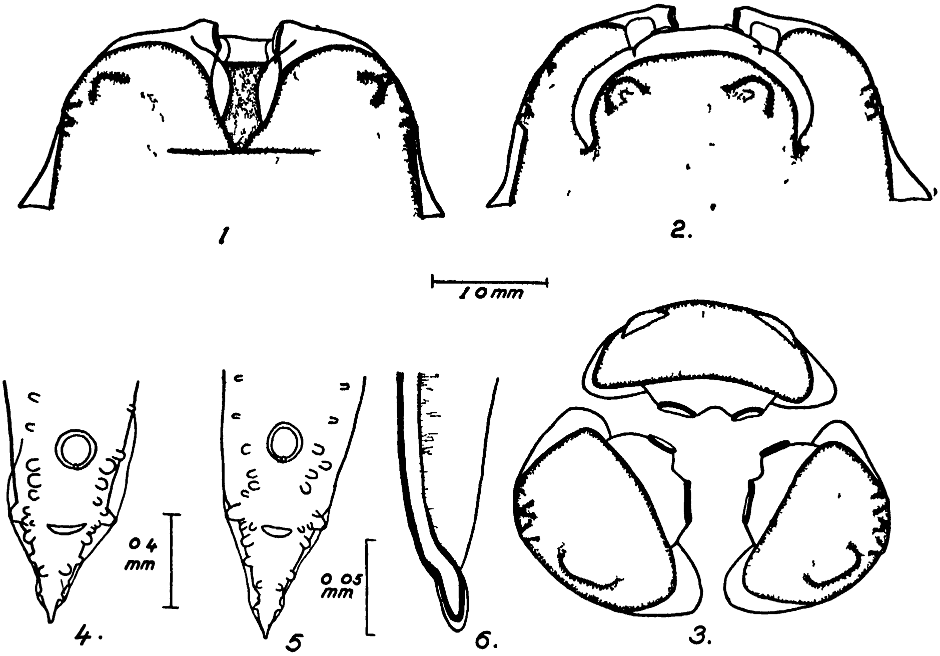
Specific description. There is a great variation in the length of *A. columbae* as given by different authors. The length ranges from 16–25 mm. for the males and 20–35 mm. for the females, as stated by Neumann and MacQueen (23), to 60–70 mm. for the males and 70–90 mm. for the females, as stated by Baylis and Daubney (6). The lengths noted by other authors fall between these two extremes. It should be noted that this parasite occurs in a variety of hosts, and Cram (7) suggests that the variation in length may be associated with this fact. In my specimens the males range from 35 to 42 mm., the females from 43 to 54 mm.

At the anterior extremity are two semi-elliptical membranes, projections of the cuticle. They are about the same length as the pharynx and are marked for part of their width with continuations of the striations of the body cuticle. The pharynx is 2.5 to 2.62 mm. long.

The number of caudal papillae in the male has been given by different authors as 10 to 14 pairs. Johnston (15) figures 13 on one side and 14 on the other. Irwin Smith (14) figures 13 pairs, Yamaguti (31) 13 pairs, and Baylis and Daubney (6) 14 pairs. In the material studied 14 pairs seems to be the normal number, but it is not unusual to find papillae missing or duplicated. The normal arrangement appears to be five pairs of post-anal papillae, of which the third pair is small and inconspicuous and in certain individuals appears to be lacking, a condition which Baylis and Daubney also noted. There is an adanal group of four pairs, one large lateral and three smaller, more ventral pairs. No changes in the number of these papillae were noted, but there are slight variations in different individuals in the relative positions of the three more ventral pairs. Between the anus and the genital sucker are three pairs of rather large papillae. It is not uncommon to find an extra papilla, that is, four on one side and three on the other, and in one specimen there are only two on one side and three on the other. Near the level of the anterior margin of the sucker is a pair more ventrally placed, and anterior to that is found the final pair. The last two pairs mentioned are quite small and inconspicuous. In one of the specimens examined the last pair appeared to be missing; according to Baylis and Daubney, it may be duplicated.

In the male, the anus is 0 44 to 0 48 mm. and the posterior margin of the genital sucker 0 58 to 0.72 mm. from the tip of the tail. The genital sucker varies in outline from circular to sub-circular and sizes noted were 176 × 176μ, 184 × 175μ and 176 × 169μ. The spicules varied from 1 05 to 1.72 mm. in length and were 57μ wide. The proximal part is slightly expanded and the distal extremity tapers abruptly to a blunt point; the posterior part has a cuticular expansion on the inner surface (Fig. 6).

In the female the anus is 0 9 to 1 1 mm. from the tip of the tail.



FIGS 1–6 *Ascaridia columbae* FIG 1 *Head, ventral view* FIG 2 *Head, dorsal view* FIG 3. *Head, front view.* FIGS 4 AND 5 *Caudal papillae of male.* FIG. 6. *Tip of spicule.*

Although the lip structures are of considerable taxonomic importance they have been accorded little attention in *A columbae*. The lips are similar to those of *A. galli*, as described by Ackert (1), that is, three prominent lips, one dorsal and two subventral, the distal margins of each being divided into three lobes, one medium and two lateral. However, where in *A. galli* there is a dentigerous ridge on the inner surface of each medium lobe, in *A. columbae* the inner surface has two saucer-shaped sucker-like structures, placed in such a position that there are three pairs, the members of each pair directly opposing one another (Figs. 2 and 3). They probably function as a hold-fast organ. In reference to the labial papillae, Johnston (15) mentions two small papillae on the dorsal lip. Yamaguti (31) states that, "the three lips are 0 07 to 0.11 mm. long and bear each two papillae, those of the dorsal lip being smaller and inconspicuous." A careful study of the labial papillae showed that there are two large papillae on the dorsal lip (Fig. 2); on each of the ventral lips there is one large, ventrally placed papilla, and a group of three smaller ones more dorsally placed (Fig. 1); in one specimen the dorsal group was found to consist of four papillae. This suggests that the number of this group may vary, as in the case of the caudal papillae of the male.

Very characteristic of *A. columbae* is the presence of large vesicular-like structures that fill the interior of the body of both sexes. Dujardin and Irwin Smith who appear to be the only authors to mention them, refer to them as "orbicular corpuscles" and consider them to be present only in the female.

Baylis and Daubney (6) mention 26 to 30 pairs of cervical papillae. I have been unable to recognize them.

The eggs vary in size from 78 to 84μ long by 49μ wide.

Capillaria Zeder, 1800

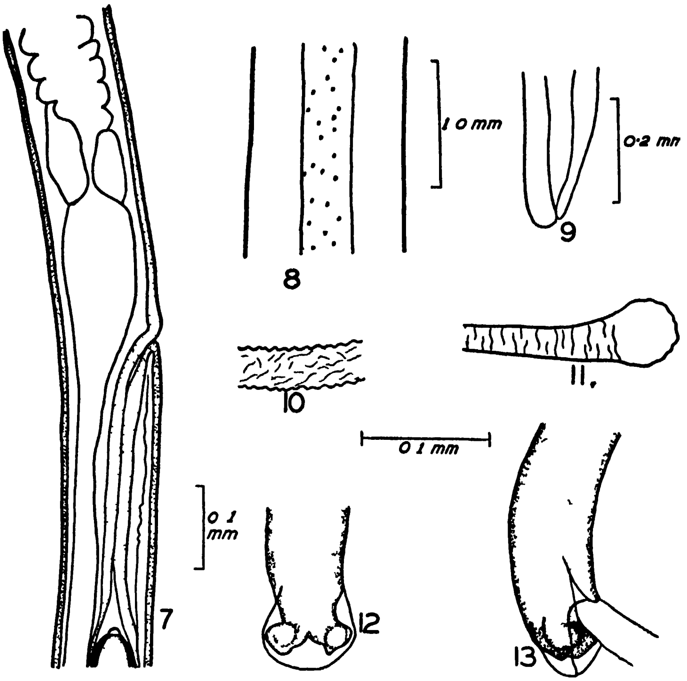
Generic diagnosis (From York and Maplestone). Body capillary; mouth simple; cuticule with bacillary bands dorsal, ventral or lateral in position; oesophagus long and gradually increasing in size posteriorly. Male: anus terminal or sub-terminal, small membranous caudal alae or bursa-like structure present or absent; spicule long and slender, with or without spines on its surface. Female: vulva near termination of oesophagus. Oviparous eggs lemon shaped, with the usual opercular plugs at the poles.

C. columbae Rudolphi, 1819

Specific description. Long thread-like worms with a white translucent appearance; cuticle finely striated transversely; two broad lateral bacillary bands which thin out at both ends, the surface of the bands being irregularly dotted with black spots; cells of the oesophagus rectangular, annulated, with a prominent nucleus in the centre; at the region where the oesophagus and the intestine meet are two smaller non-annulated cells on each side of the digestive tube (Fig. 7).

Male: Only one entire male was recovered. It measures 11.4 mm. in length. According to other authors the length may vary from 8.1 to 11.7 mm. The oesophagus is slightly less than half the entire length or 5.53 mm. The cloacal aperture is nearly terminal and is surrounded by a membranous bursa-like structure. Supporting this membrane are two broad rays, each of which appears to have a secondary outgrowth (Figs. 12 and 13). The spicule in two specimens measures 1.56 mm. and 1.4 mm.; it has a blunt, rounded point and at the proximal extremity expands like a trumpet (Fig. 11). In the entire male specimen the spicule sheath is extruded and measures 2.94 mm. It is irregularly annulated and covered with fine markings (Fig. 10). At the widest part it measures 0.034 mm.

Female: Three females measured 14 mm., 15.1 mm. and 19 mm. This is greater than the lengths recorded by other authors. Graybill (13) mentions 10 to 12.3 mm., Irwin Smith (14), 13 to 16.24 mm. and Morgan (21) 10.1 to 14.5 mm. as the lengths for the females of *C. columbae* they examined. In two females the oesophagus is slightly more than one third of the total length, or 39%. The posterior extremity tapers to a blunt point with the cloacal aperture slightly sub-terminal (Fig. 9). The vulva is situated near the junction of the oesophagus and the intestine, and has slightly protruding lips (Fig. 7). The vagina passes backwards.



FIGS. 7–13. *Capillaria columbae.* FIG. 7. *Female, in region of vulva; lateral view.* FIG. 8. *Lateral view showing bacillary band* FIG. 9. *Posterior extremity of female.* FIG. 10. *Anterior end of spicule* FIG 11. *Spicule sheath.* FIG 12. *Posterior extremity of male, ventral view.* FIG 13. *Posterior extremity of male, lateral view*

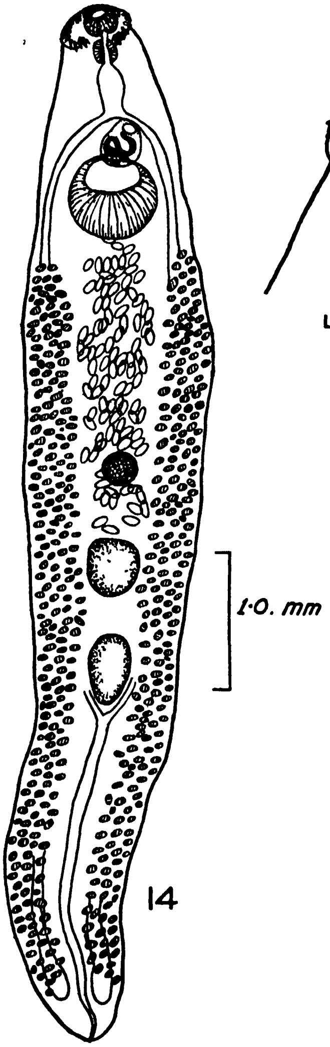
The eggs are ellipsoid, brownish in color but often appear greenish when collected in the feces. They have a thick shell discontinued at either pole where two opercular plugs are present. Egg sizes vary from 47 to 72μ long and 24 to 31μ wide.

Echinostoma paraulum (Dietz, 1909)

This appears to be the first time *E. paraulum* has been reported in North America.

In 1925 Zunker (35) described this parasite from the pigeon as new under the name *Echinostoma columbae.* Later Monnig (20) states it to be a synonym of *Echinopharyphium paraulum* Dietz, 1909. Unfortunately I have been unable to see the original description by Dietz, but the characteristics revealed by my specimens, although resembling Zunker's description, would not place them in the genus *Echinoparyphium.* According to Dietz (10) ". . . . im Gegensatz zu allen andern von mir unterschiedenen Echinostomidengattungen sind jedoch die Randstacheln beider Reihen nicht gleich gross, sondern die Stächeln der oralen Reihe kleiner als die der aboralen." While it is true that on the average the aboral spines are longer than the oral spines the difference is very slight, and as there is an overlapping of sizes, I believe that the trematode should be kept in the genus *Echinostoma*. Therefore, as I consider *Echinostoma columbae* to be a synonym of the echinostome described by Dietz, the name should be changed to *Echinostoma paraulum*.

Specific description. Elongate worm measuring from 4.7 to 8.5 mm. in length. In my specimens the greatest width occurs immediately posterior to the acetabulum and varies from 1.06 to 1.3 mm. In Zunker's figure the greatest width is in the posterior part of the body. The head crown bears two ventral groups of five spines, the arrangement of which is not constant,



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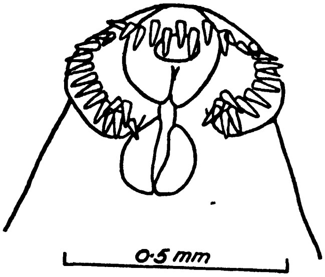

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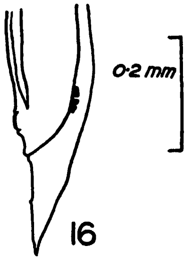

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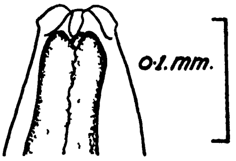

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FIGS. 14–15. *Echinostoma paraulum*. FIG. 14. *Ventral view*. FIG. 15. *Ventral view of anterior end, showing head crown.*

FIGS. 16–17. *Ascaridia columbae*. FIG. 16. *Posterior extremity of third stage male larva*. FIG. 17. *Anterior extremity of third stage larva.*

and 27 marginal spines (Fig. 15). On the dorsal surface the spines tend to form two rows, and the length of the aboral spines varies from 68 to 80μ while those of the oral are from 66 to 70μ. No spines are found on the body, but, as has been suggested by Mönnig (20) in reference to Zunker's specimens, they may have been lost during post mortem changes. The oral sucker is subcircular and is 0.17 to 0.22 mm. in diameter, the ventral sucker 0.52 to 0.64 mm. and the pharynx 0.17 to 0.22 mm. The oesophagus is 0.36 mm. long and bifurcates halfway between the pharynx and acetabulum. The two digestive caeca reach to near the posterior tip of the body.

The testes are usually oval to circular in outline but may be lobed, tandem, and lie in the third quarter of the body. Zunker (35) states that in the mature specimens he examined, the testes were constricted in the middle. The cirrus pouch is found between the point of bifurcation of the oesophagus and the acetabulum, usually partly overlapping the latter. The ovary is subcircular, located just anterior to the anterior testes. The uterus is a long convoluted tube. The vitellaria are extensive and reach from the posterior border of the acetabulum to near the posterior end of the body. The eggs are oval, and are 96 to 98μ long by 56 to 64μ wide.

The excretory system is Y-shaped with a long narrow base bifurcating immediately caudad of the posterior testis, the two branches running forward to the posterior border of the acetabulum.

In general, the characters as described above agree quite consistently with those stated by Zunker. The differences in shape of body and outline of the testis are not constant characters and therefore could not form the basis for the description of a new species.

Check List of Parasites of Pigeons

Parasite	Distribution
Trematoda	
STRIGEIDAE	
Apatemon gracilis Rudolphi, 1819.	?
HARMOSTOMIDAE	
Brachylaemus commutatus Diesing, 1855.	South Europe, North Africa, England, Indo-China.
B. mazzantii Travassos, 1917.	Brazil.
ECHINOSTOMIDAE	
Hypodaraeum conoideum Bloch, 1782.	Europe.
Echinoparyphium recurvatum Linstow, 1873.	Europe.
Echinostoma paraulum Dietz, 1909.	Europe, Java, Canada.
E. robustum Yamaguti, 1935.	Japan.

Check List of Parasites of Pigeons—*Concluded*

Parasite	Distribution
Cestoda	
BOTHRIOCEPHALIDAE	
Diphyllobothrium erinacei (Rudolphi, 1819), 2nd stage larvae.	?
ANOPLOCEPHALIDAE	
Aporina delafondi Railliet, 1892.	Asia, Africa, Europe, South America, North America.
DILEPIDIDAE	
Choanotaenia infundibulum Bloch, 1779. ?.	World-wide.
HYMENOLEPIDAE	
Hymenolepis (Hymenolepis) serrata Fuhrmann, 1906.	Europe.
H. (Hymenolepis) serrata var. *birmaniea* Meggitt, 1924.	India.
H. (Weinlandia) columbae Zeder, 1800.	Europe, India, North America.
DAVAINIIDAE	
Cotugnia cuneata var. *tenuis* Meggitt, 1924.	Burma, India, Egypt.
C. cuneata var. *nervosa* Meggitt, 1924.	Burma, India, Japan.
Raillietina (R.) micracantha Fuhrmann, 1909.	Africa, Europe, Asia.
R. (R.) nagpurensis Moghe, 1925.	India.
R. (R.) torquata Meggitt, 1924.	Burma.
Raillietina Skrjabinia bonini (columbae) (Megnin, 1899).	England, Europe.
R. (R.) clerci Fuhrmann, 1920.	Asia, Africa.
R. (R.) joyeuxi Lopez Neyra, 1929.	Spain.
R. (R.) tunetensis Joyeux & Houdemer, 1928.	Tunis, India.
R. (R.) taiwanensis Yamaguti, 1935.	Japan.
R. (R.) echinobothridia Megnin, 1881.	Cosmopolitan.
R. (R.) volzi Fuhrmann, 1905.	Asia, Sumatra.
R. Fuhrmanetta crassula Rudolphi, 1819.	Europe, Africa, North America, South America.
Nematoda	
HETERAKIDAE	
Ascaridia columbae Gmelin, 1789.	North America, South America, Europe, Asia, Africa, Australia.
SPIRURIDAE	
Acuaria (Dispharynx) spiralis Molin, 1858.	North America, South America, Europe, Asia, Africa.
Tetrameres (Tetrameres) fissispina (Diesing, 1861) Travassos, 1914.	North America, Europe, Asia, Africa.
T. (T.) confusa Travassos, 1919.	South America.
TRICHINELLIDAE	
Capillaria columbae Rudolphi, 1819.	North America, South America, Asia, Africa, Australia.
FILARIIDAE	
Eulimdana clava Wedl, 1855.	Europe.
E. mazzantii Railliet, 1895.	Europe.
STRONGYLIDAE	
Syngamus trachealis Siebold, 1836 ?	Europe.
TRICHOSTRONGYLIDAE	
Ornithostrongylus quadriradiatus Stevenson, 1904.	North America, Australia, Cuba, Natal.

The following helminths have been experimentally introduced into the domestic pigeon.

TREMATODES:— *Brachylaemus fuscatus, Echinostoma erraticum, E. exile, E. parvum.*

NEMATODES:—*Oxyspirura parvovum, Tetrameres (Microtetrameres) helix, Trichinella spiralis.*

Acknowledgments

This investigation was carried out under the supervision of the Director of the Institute of Parasitology. It is with pleasure that I express my thanks to the Director and the staff, for their invaluable advice and assistance. I would also like to express my appreciation to Messrs. Henry Gatehouse and Son for supplying pigeon material and to Mr. Louis Meyer for supplying the living pigeons used during the course of the investigation.

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THE EXPERIMENTAL INFECTION OF PIGEONS AND POULTRY WITH *ASCARIDIA* AND *HETERAKIS*[1]

BY M. J. MILLER[2]

Abstract

The results of investigations on the intertransmission of pigeon and fowl parasites are presented. They show that *Heterakis gallinae* and *Ascaridia galli* from the fowl are not transmissible to pigeons. Larval *Ascaridia columbae*, however, will develop in young chicks to a 96-hour stage, although they are not present 120 hours after infection.

It is not uncommon for species of parasites known to infect pigeons to be found infecting other domestic birds. It is interesting, however, to note that the cestodes of pigeons are rarely, if ever, found in any of the other domestic birds.

As far as is known very little work has been done on the experimental infection of domestic birds with pigeon parasites or *vice versa*. The only record found was a successful infection of poultry with *Capillaria columbae* from the pigeon (2). Consequently attempts were made to introduce *Ascaridia columbae* into the fowl, and at the same time, *A. galli* and *Heterakis gallinae* into pigeons.

The eggs of the pigeon parasites were collected from the feces of pigeons confined in cages in the Institute of Parasitology, and from gravid female parasites removed after autopsy. *H. gallinae* were obtained from fowl intestines supplied by Messrs. Henry Gatehouse and Son. In the case of *A. galli*, a single gravid female recovered from a fowl egg provided a sufficient supply of eggs.

The pigeons were all examined on arrival for internal parasites, and those used in the experiments were given three successive fecal tests, at intervals of 48 hours, before the date of infection. If, after these tests, the feces showed no trace of helminth eggs, the bird was considered to be free of helminths.

The chicks used in the experiment were secured from the Macdonald College Poultry Department on February 5 as day-old chicks, and were placed in a brooder, all parts of which had been thoroughly sterilized with steam. Later they were moved to wire cages, each cage containing only those birds concerned in one particular experiment. All experimental birds were banded to eliminate confusion in identification.

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Contribution from the Institute of Parasitology, McGill University, Macdonald College, Que., with financial assistance from the National Research Council.

[2] *Graduate Student.*

Two methods were employed in the culturing of parasite eggs. In one an antiseptic substance was added to an aqueous egg culture to keep down molds and bacteria, and in the other, a lowered humidity was used for the same purpose. Both methods proved to be satisfactory.

The infective eggs to be fed were drawn off with a pipette and deposited on a filter paper folded in a funnel. In this way the liquid passed through, while the eggs remained on the filter paper. The part of the filter paper bearing the eggs was then cut out, mixed with chick mash, and rolled into a small pellet. With the aid of forceps this pellet could easily be placed in the throat of the bird, which quickly swallowed it. Towards the end of the investigation the filter paper with the eggs on it was fed without mixing with the mash. This was a much quicker method and quite as efficient. *A. columbae* eggs, which were collected in the feces, were passed through fine bolting silk. This separated the eggs from the larger fecal particles. The eggs were then fed in the manner described above.

EXPERIMENT 1

Artificial Infection of Pigeons with A. galli

On January 29, a gravid female of *A. galli* was dissected; the uterus was removed and teased out on a plate of glass which was placed in a closed jar at room temperature, with a relative humidity of 97.5%. By February 9, vermiform larvae could be seen moving within the shell. Whether the eggs were infective could not be determined, but in order to make sure, they were left considerably longer. On February 24, a fungus growth was noticed on the eggs, which were then washed off and stored in a small Petri dish with about 1% formol solution. This killed the fungus.

On February 26, two pigeons, which had been previously proved ascaridia-free, were fed about 150 infective ascaridia eggs. Infection was repeated on March 1, 4, 8, 11, 15, 18 and 22. At the same time, in order to determine whether the eggs were infective, two chicks, approximately three weeks of age, were removed from the brooder and placed in a small cage away from the others. In order to take into consideration the possibility of an age immunity in pigeons to ascaridia, two pigeons known to be ascaridia-free were infected with eggs of *A. columbae*, and the infection repeated on the same dates as the infection with *A. galli*. Two ascaridia-free pigeons were used as controls.

On March 24, one of the pigeons infected with *A. columbae* was killed and the intestinal contents thoroughly examined. In the posterior portion of the gut one small larva was found. This was presumably a 48-hour larva, that corresponding with the time since the last infection; it also corresponded to the 48-hour larvae found in other experiments.

The other pigeon infected with *A. columbae* was killed on March 26, having been fed infective eggs 17 hours previously; again only one larva was recovered. These results suggest a strong age immunity in pigeons to *Ascaridia columbae*. This age immunity would probably affect *A. galli* as well, and therefore, although on March 28 no parasites were found in the pigeons that had been infected with *A. galli*, the possibility is not excluded that young squabs may be susceptible to infection by *A. galli*.

In the examination of the control chicks infected with *A. galli*, some interesting results were noted. The first chick killed on March 26, yielded 11 larval ascaridias, and while according to Ackert (1) the average length for a 29-day *A. galli* is about 31 mm., these varied in length from less than a millimetre to 9 mm. It first appeared as though the larvae were not the result of the original infection but were due to an infective contamination from some outside source. However, as the birds were kept under strictly sanitary conditions, and as none of the control birds showed the infection, the larvae must have been hatched from the eggs fed on February 26. The other chick infected at the same time was killed on April 16, to permit the parasite to reach maturity and thus make identification possible. Two male ascaridias were recovered from this chick, and here also, there was a considerable difference in their sizes, one measuring 42 mm. in length and the other 30 mm. Although the ascaridias were identified as *A. galli* they were only about three fifths of the normal length and the spicules were not fully formed. This lack of development may be due to some form of immunity reaction set up by the chicks, that interferes with the normal growth of the parasites, which are affected in an unequal manner. These results are quite different from any published by other workers in reference to the life history of *A. galli*.

EXPERIMENT 2

Artificial Infection of Pigeons with Heterakis gallinae

On January 21, several hundred female heterakis were teased out on a plate of glass and incubated in a relative humidity of 97.5%. On January 31, a heavy growth of fungus was noticed on the eggs, and a subsequent examination showed that those eggs teased out of the uterus and exposed contained air bubbles and were dead. The eggs protected by the uterine wall however, were still alive, and when examined on February 8, contained well developed larvae. It would seem that the fungus growth is lethal to the unprotected egg. The eggs were washed off into a small Petri dish and stored in a solution of 1% formol.

Only one pigeon was infected in this experiment and on February 27 it was fed about 125 eggs. The infection was repeated on March 1, 4, 8, 11, 15, 18 and 22. At the same time, in order to check the infectivity of the eggs, a chick was removed from the brooder, infected, and isolated in a cage. On March 23, 24 hours after the last infection, the pigeon was killed and the gut contents examined. Although a very thorough examination was made, no heterakis of any stage were recovered, nor were any eggs found. To determine whether the eggs were infective the control chick was killed, and on examination it yielded four adult heterakis. While this is not a large percentage of infection, it does show that some of the eggs were infective. These results suggest the conclusion that *Heterakis gallinae* is not infective to adult pigeons. The possibility that it may be transmitted to young squabs, however, should not be overlooked.

What happens to the eggs when they enter the pigeon is an interesting point, and when we take into consideration the fact that no larvae or even eggs were found in the bird, the most likely explanation seems to be that the eggs hatch and the larvae, finding their environment incompatible, die and are digested by the host. There is the possibility that the eggs do not hatch at all and are passed out with the feces within 24 hours, or that the eggs do hatch and the larvae, not finding any caeca, pass on out. It seems unlikely however that they should completely pass through the alimentary canal in 24 hours. Moreover, as no larvae were ever found in the feces, the last explanation is unsatisfactory.

EXPERIMENT 3

Artificial Infection of Fowl with Ascaridia columbae

On December 20, the first attempt was made to incubate *Ascaridia columbae* eggs; they were placed in a shallow solution of 1% potassium dichromate. When examined on December 31, most of them contained active larvae. Again on January 15, eggs were sedimented on a plate of glass and sealed in a jar in 100% humidity. By January 25, although a fungus growth had appeared on the eggs, they nearly all contained active larvae. While the larvae appeared well developed, they were probably not infective. On February 23, eggs were sedimented on a plate of glass in a relative humidity of 90%. On February 25, another group of eggs was cultured in 1% solution of formol. This culture seemed to progress more rapidly, and although started two days later, reached the vermiform stage sooner than did the first mentioned culture. Eggs from the culture of February 25 were used in this experiment.

On March 18, ten chicks were each fed 1000 embryonated *Ascaridia columbae* eggs. On March 28, ten days after infection, one of the infected birds was killed, and a careful examination of the intestinal contents failed to reveal any ascaridia larvae. In order to find what happened to the *Ascaridia columbae* eggs after being swallowed by the chicks, the chicks were all reinfected on March 30, and individuals were killed and examined at 8, 24, 48, 72, 96, 120 and 144 hours after infection. At the same time, the feces were examined for eggs and larvae at intervals from one to 46 hours after infection.

As the larvae are very small, great care was taken in the examination of the intestinal contents of the birds. The gut was divided into its different parts—oesophagus, stomach, duodenum, small intestine, caeca and large intestine—and each part was opened up and individually shaken in water. The liquid was poured off and allowed to settle, while the mucosa was carefully examined for nodules or haemorrhagic spots. Any suspicious looking areas were scraped on to a compressorium and examined microscopically.

The liquid was allowed to settle for a considerable length of time, the upper fluid was poured into another vessel and allowed to settle in turn. This was repeated three or four times to make sure that no larvae were poured off with the upper fluid. The residue in each case was carefully examined under a binocular microscope. When larvae were found they were transferred to a slide, fixed by passing the slide over the flame of a spirit lamp, and studied under a microscope. The caeca of the chicks infected for more than 72 hours were not examined, and only in the eight-hour infection was any part above the duodenum examined.

In the chick killed eight hours after infection, eggs were recovered in the crop and the stomach. No larvae were found in any part of the gut.

The chick killed 24 hours after infection had numerous larvae in the small intestine; none was found in any other part of the gut, or in the immediate feces. Apparently the eggs hatch somewhere between eight and 24 hours after infection.

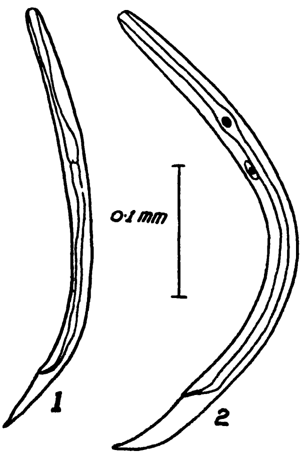
Description of the Larvae (Figs. 1, 2)

Relatively thick-bodied larvae; parallel sided body becoming somewhat thinner posteriorly, with a pointed tail; length 0.34 mm., width at widest part, distal portion of oesophagus, 0.018 mm.; oesophagus 0.12 mm. long or a little more than one-third body length, distal portion slightly expanded; intestine narrow, leading to an anus with slightly prominent lips, 0.049 mm. from tip of tail; the interior of the body filled with granular material.

The chick killed 48 hours after infection yielded five larvae from the small intestine. Here again, no larvae were found in any other part of the gut or in the immediate feces. The length in this case was 0.354 mm., the oesophagus 0.126 mm. and the distance from the tip of the tail to the anus the same as in the 24-hour larvae. These larvae were similar to the 24-hour one in all other respects except that here an excretory pore and duct could be made out 0.088 mm. from the anterior tip of the body.

The chick killed 72 hours after infection harbored five larvae in the posterior part of the gut. The larvae in this case were very similar to those obtained from the chick infected 48 hours, but were slightly longer.

The chick killed 96 hours after infection had four larvae in the posterior part of the small intestine and one in the large intestine. The larvae were approximately 0.39 mm. long, and were proportionately broader than the 24-hour larvae, the width being 0.023 mm. The oesophagus had a shorter and more distinct posterior bulb but was practically the same length. A short distance behind the bulb of the oesophagus was what appeared to be a genital anlage. The distance from the anus to the tip of the tail was 0.06 mm. (Fig. 2).



FIGS. 1 AND 2. *Ascaridia columbae, larval stages.* FIG. 1. *24 hours after infection.* FIG. 2. *96 hours after infection.*

No larvae were detected in the chicks killed 120 and 144 hours after infection.

The examination of the feces failed to reveal any eggs or larvae before 24 hours after infection. From 24 to 34 hours, larvae about 0.34 mm. long were recovered. No eggs were ever found, nor were any larvae found after 34 hours.

It would appear, therefore, that the eggs of *Ascaridia columbae* hatch in the chick between 8 and 24 hours after infection, and from 24 to 34 hours the great proportion of them pass out with the feces. However, some remain in the small intestine until between 96 and 120 hours after infection, when they die and presumably pass out with the feces or are digested by the host.

Acknowledgments

This investigation was carried out under the supervision of the Director of the Institute of Parasitology. It is with pleasure that I express my thanks to the Director and the staff for their invaluable advice and assistance.

I would also like to express my appreciation to Messrs. Henry Gatehouse and Son for supplying pigeon material and to Mr. Louis Meyer for supplying the living pigeons used during the investigation.

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A NOTE ON THE REDUCTION IN THE FECUNDITY OF *TRIBOLIUM CONFUSUM* DUE TO EATING ITS OWN EGGS[1]

By John Stanley[2]

Some time ago (1) the writer showed that the growth of the egg population of *Tribolium confusum* could be described by an expression

$$T = \frac{c}{a} N_2 + \frac{ad - bc}{a^2} \log_e \left(\frac{aN_2 + b}{b} \right)$$

and this curve was figured in (1).

On making an attempt to produce this curve experimentally by removing the old eggs every four days and replacing them by an equivalent number of new young eggs, a curve of roughly similar form was obtained for the early stages, with evidence of a later periodic fluctuation instead of an approach to an asymptote.

Further experimental work, as well as mathematical analysis of the situation, shows that the curve actually obtained is not nearly as steep as it should be if the egg-laying rate remains constant, and there is good evidence to show that the eating of eggs reduces fecundity at a later time. This effect can be shown to be competent not only to flatten the curve but also to produce the periodic fluctuations that occur. A full description of this matter will be made in a later publication.

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[1] *Manuscript received February 22, 1937.*
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RAPHIDASCARIS LAURENTIANUS SP. N. (ASCAROIDEA) FROM *SALVELINUS FONTINALIS* (MITCHILL) IN QUEBEC[1]

BY L. R. RICHARDSON[2]

Abstract

A new Raphidascarid nematode, *Raphidascaris laurentianus* is described from material collected from the intestine of the speckled trout. The specimens were obtained from Labelle County, Quebec. *R. laurentianus* is of moderate size and is characterized by having delicate caudal alae, parallel uteri, and only five to twelve pairs of preanal papillae. This is the first definite record of such a nematode from speckled trout.

The records of nematodes parasitic in the speckled trout (*Salvelinus fontinalis* (Mitchill)) in eastern North America have been few. Within recent years several new species have been recorded. Skinker (3) has described *Metabronema canadense* from a single specimen obtained from a trout taken at the Matamek River, Quebec. Chandler (1) has described *Cystidicoloides harwoodi* from specimens taken from the stomach of trout collected near Elizabethtown, northern New York state, while Simon and Simon (2) have described *Philonema agubernaculum* from speckled trout and other hosts in the Wyoming area.

An examination of the intestines of six speckled trout collected at Lac Sept Frères, Labelle County, Quebec, in June of 1933, during the course of a biological survey conducted by the Department of Public Works, Game and Fisheries, showed the presence of large numbers of a new Rhapidascarid nematode. The worms were present throughout the length of the intestine with the exception of the rectum and the stomach. As many as 75 worms, ranging in length from 5.0 mm. to 29.0 mm. and representing both sexes, were taken from the gut of a single trout. The majority of the worms were present in the pyloric caeca. The largest females were collected from the lower portion of the intestine. Immature specimens were common and ranged in length up to 10.0 mm.

The hosts were also heavily infected with the trout tapeworm (*Eubothrium salvelini* (Schrank, 1786)) and with the small coronate trematode *Crepidostomum fausti* Hunninen and Hunter 1933.

The present specimens are located within the genus *Rhapidascaris* on the basis of the arrangement of the alimentary canal and associated structures. The presence of a ventriculus and ventricular appendix, and the absence of other caeca, the cuticular expansions of the lips and absence of dentigerous ridges, are characters descriptive of this worm and diagnostic for this genus. The presence of caudal alae does not seem to have been noted in other worms of the genus and may serve to set aside the present specimens from previously described species in the genus.

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[1] *Manuscript received March 12, 1937.*
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[2] *Assistant, Department of Zoology, McGill University.*

Raphidascaris laurentianus
SP. NOV.

In the present collection the females reach a maximum length of 29.0 mm. with a maximum diameter of 1.08 mm. at the beginning of the posterior third of the body, and a diameter of 0.72 mm. at the level of the vulva. The average female specimens are from 17.0 mm. to 25 0 mm. in length. In these the posterior portion of the body is slightly wider than the anterior, and the diameter of the body increases gradually from the narrow head back to the level of the vulva where it varies from 0 52 mm. in the smaller specimens to 0 70 mm. in the larger. Behind this point, the body is of practically uniform diameter nearly to the level of the anus. With the development of large numbers of eggs in the uteri and median vagina, the posterior portion of the body becomes obviously swollen. The tail is long, sharply pointed and tapering gradually from just before the anus to extend 0.5 mm. beyond that point. The cuticle is striated, there being five to seven striations in a length of 0.1 mm. The cuticular ridges frequently present a retrorse appearance. Symmetrical lateral cephalic alae are present. These are narrow, 0.12 mm. in breadth at their widest point, and extend backwards from the neck to a point just beyond the

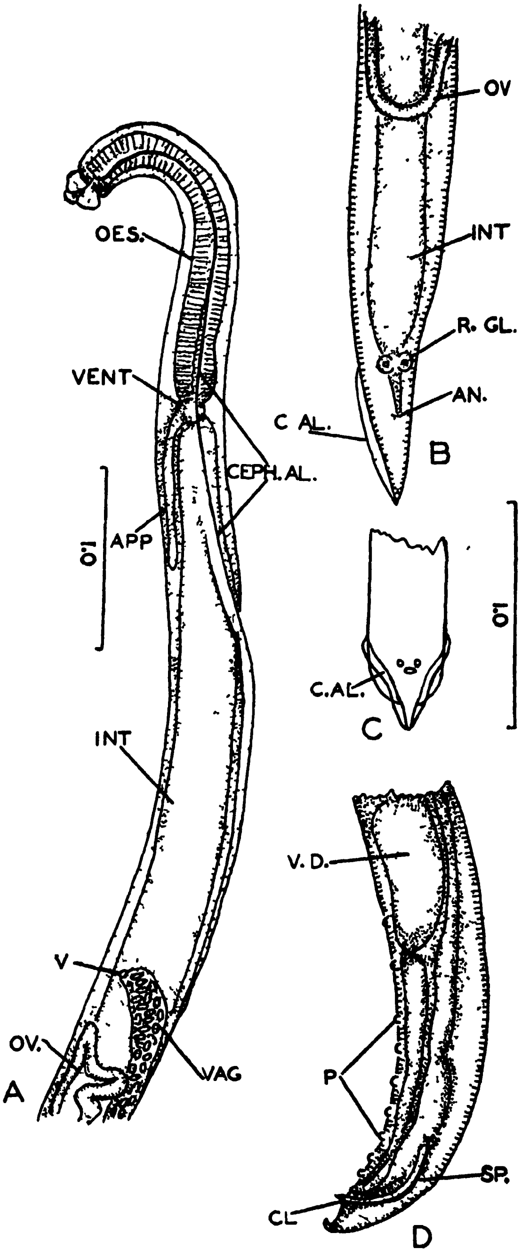

FIG. 1. *A. Anterior third of adult female, 20.0 mm. long, drawn in ventro-lateral view. B. Posterior end of adult female in ventro-lateral view. C. Outline view of posterior end of male drawn from the ventral aspect. D. Posterior end of male, lateral view. All scales in mm.* AN., *anus;* APP., *ventricular appendix;* C.AL., *caudal alae;* CEPH. AL., *cephalic alae;* CL., *cloaca;* INT., *intestine;* OV., *ovary;* P., *papillae;* R. GL., *rectal glands;* SP., *spicule;* VAG., *vagina;* V.D., *vas deferens;* VENT., *ventriculus;* VULV., *vulva.*

vulva. Delicate short caudal alae are present and difficult to detect. They extend from a point 0.5 mm. before the anus to the tip of the tail. The caudal alae are 0.030 mm. in width. There are no sulci on either the cephalic or the caudal alae. There are three lips, one dorsal and two subventral. The dorsal lip has two papillae, the ventral lips one each. The border of each lip is emarginate. The oesophagus in the female is 2.0 mm. to 3.4 mm. in length and averages $\frac{1}{8}$ of the total length of the body. The ventriculus is 0.15 mm. to 0.17 mm. in length and is wider than long. The thin-walled ventricular appendix is 0.9 mm. to 1.1 mm. in length. The gut is simple, expanded throughout its length and is joined to the anus by a short, narrow tube with delicate walls. There is no intestinal caecum. The vulva is situated at a point distant $\frac{1}{3}$ the total length from the anterior end of the body. The vulva is not protected by prominent lips. The median sac-like vagina is elongate and terminates posteriorly in two simple uteri. The latter are parallel, not divergent, and pass straight backwards to a point 1.5 mm. from the anus where they turn forwards into the oviducts. The latter pass forward nearly to, but not reaching the level of the vulva and then return as the ovaries. The ovaries are greatly convoluted with the exception of the posterior portion, which terminates before reaching the anus. The ovaries are lateral to and conceal the other portions of the reproductive system. The ova removed from a gravid female are subspherical and have a diameter of 0.080 mm.

The male is smaller than the female and reaches a maximum length of 23.6 mm. with a diameter of 0.78 mm. The majority of the mature males in the present collection are 17.0 mm. to 22.0 mm. in length. The body is of an almost uniform diameter posterior to the beginning of the second third of its length, but tapers gradually forwards from this point to the narrow head. The posterior end of the body is bent ventrally into a semicircle. The body terminates more abruptly than in the female, posterior to the cloaca the short tail taking the form of a small high cone. The mouth parts are similar to those of the female. The cuticle is striated. Symmetrical lateral cephalic and caudal alae are present and similar to the same structures in the female. The cephalic alae extend to the level of the anterior coils of the testis. The cuticle bears five to twelve pairs of low, non-pedunculate preanal papillae and one pair of adanal papillae. The number increases in the larger males, some of which also have two or three pairs of incompletely developed papillae anterior to the normal ones. The oesophagus is $\frac{1}{6}$ to $\frac{1}{10}$ the total length of the body. The central canal is corrugated. The ventriculus has a longitudinal diameter $\frac{1}{12}$ that of the length of the oesophagus. The appendix and gut are the same as in the female. The testis is convoluted and reaches forward to the posterior end of the ventricular appendix. The coils of the testis are wider than long, close and more regular than the coils of the ovary in the female. The testis terminates in a median, elongate vesicula seminalis. The spicules are equal, curved and 0.45 mm. to 0.70 mm. in length. Each spicule bears a wing in the form of a gutter. The shaft of the spicule is 0.016 mm. in diameter, the wing 0.044 mm. in width. The spicules terminate with an obtusely pointed distal end.

The present specimens are distinct from *R. brachyurus* Ward and Magath, 1916 (5) in the presence of papillae on the tail of the male. They differ from *R. cayugensis* Wigdor, 1918 (6) in having the uteri parallel and not divergent at their entry into the median vagina. *R. laurentianus* is distinct from *R. canadensis* Smedley, 1933 (4) in having the cephalic alae equal, and lacking a sulcus; a smaller number of papillae on the caudal end of the body in the mature male, a more posterior position of the vulva in the female and in having eggs of a larger size. The present specimens are smaller worms than *R. acus* (Block, 1779).

I have examined the viscera of several northern pike (*Esox lucius* Linn.) taken from lakes in Labelle County, in the expectation of obtaining specimens of *R. canadensis* for closer comparison with the present specimens, but so far without result. These pike were all infected with *Proteocephalus pinguis* La Rue 1911. Specimens of pickerel (*Esox niger* LeSueur) from Brome Lake, Brome County, were heavily infected with a Raphidascarid nematode the larvae of which are commonly found encysted in the liver of perch at this lake. These specimens are also distinct from any described Raphidascarid.

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THE PHYSIOLOGY OF THE SHEEP TAPEWORM, *MONIEZIA EXPANSA* BLANCHARD[1]

BY ROBERT ARNOLD WARDLE[2]

Abstract

The influence of saline media upon the longevity, water content and polysaccharide content of *Moniezia expansa* is discussed. Worms may live two or three days in balanced salines but in the majority of experiments they die between 9 and 12 hours. Addition of glucose abbreviates the longevity period in saline media. The water content is affected only slightly by balanced salines but is influenced by the concentration of sodium chloride and by the presence of sugars and amino acids; behavior in saline media cannot be correlated wholly with changes in water content. The polysaccharide content is not significantly different from mammalian glycogen, constitutes 0.35 to 5.25% of the fresh weight, remains stable during immersion of the living worm for six hours in media that reduce muscle tonus, but decreases during immersion in media that encourage muscle tonus. Glycogen increase occurs when the medium contains glucose up to 1%, but not when the glucose content exceeds this amount or when the glucose is replaced by other sugars, by amino acids, or by glycoproteins. Saline media are adjudged unfavorable to tapeworm existence *in vitro*.

1. Introduction

The literature on the subject of tapeworm physiology is scanty and the neglect of this aspect of comparative physiology can only be attributed to the lack of established technique for keeping a tapeworm alive outside its host for a sufficient length of time to permit of physiological experimentation and observation. So closely adapted is an adult tapeworm to its habitat—which, with few exceptions, is the small intestine of some chordate animal—that its life in the relatively crude laboratory imitations of this habitat is always brief, the animal disintegrating with rapidity whether in sterile or in oligoseptic media.

The data presented below are the results of a series of experiments extending over three years with a typical tapeworm *Moniezia expansa*, employing the saline nutrient media commonly used for animal cultivation experiments, and taking as criteria of the favorableness of such media their influence upon the longevity of the tapeworm, upon its water content, and upon its polysaccharide content.

2. Material and Methods

The sheep tapeworm, *Moniezia expansa* (Rudolphi, 1810) Blanchard, 1891, was selected as a subject for this study because it is abundant and easy to obtain during the late summer in Canada, and because it has been used by

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[1] *Manuscript received April 26, 1937.*
Contribution from the Department of Zoology, University of Manitoba, Winnipeg, Canada.

[2] *Professor of Zoology, University of Manitoba.*

several other workers in this field, notably by Brand, and Cook and Sharman, the latter workers referring to their material erroneously as "*Moniezia trisonophora*". It is, however, not an ideal tapeworm for experiments of this nature since it cannot be obtained in quantity during the winter and spring, and is somewhat sensitive to removal from the host.

The tapeworms can be obtained most readily and quickly by hand-squeezing the lamb gut slowly from the pylorus to the beginning of the large intestine, and although each lamb may contain only 4–10 worms, the large scale on which lambs are slaughtered in the Canadian packing industry, and the high incidence of this worm in western Canadian lambs, permit the collection of several hundred worms per hour. Medium sized specimens, 30–50 cm. in length, that show activity and are unstained by bile, should be selected, rinsed in physiological saline, drained on soft cotton cloth, and placed as soon as possible in the experimental media. If kept at 20° C. in moist cotton cloth, however, they will live with negligible muscular activity and negligible polysaccharide and water loss up to 12 hours.

The media used were unsterilized but freshly prepared saline solutions alone or plus the addition of 1% of some nutrient substance,—sugars, amino acids, glycoproteins. Four saline bases were used in most of the experiments:

1. *Tyrodes solution*, used in single strength and two and a half times single strength; the stronger solution was used on the assumption, that later proved erroneous, that it would be nearer isotonicity for the worms than would the ordinary concentration.

2. *Phosphate saline*, which was Tyrodes solution with the sodium acid phosphate content increased from 0.02 to 0.2%, on the suggestion of Magee and Reid (10) that the presence of 0.2% of sodium acid phosphate stimulates the absorption of glucose by the intestinal mucosa of the rat.

3. *Bile saline*, a solution of 0.8% sodium chloride in water plus the addition of 10% fresh sheep bile, this concentration of bile being assumed to resemble the concentration present in the small intestine of the lamb.

4. *Sodium glycocholate*, in 1% aqueous solution, used as a ready solvent for certain nutrients (lecithin, cholesterol) not soluble in the other bases.

In the water content experiments, tests were made also with distilled water, half, double, triple, quadruple and quintuple strength Tyrodes solution; with a Ringer solution formula recommended by Brand (4); and with another Ringer solution formula recommended by Cook and Sharman (6); and with $M/5$ and $M/10$ sodium chloride solutions. These media were used in 200-ml. quantities in 250-ml. Erlenmeyer type Pyrex flasks kept in a dry incubator at 36-38° C. for a period of six hours only; five worms were used in each test and the test was repeated at least three times.

It may be noted that, throughout this paper, the term *fresh weight* means the weight of freshly collected, living material, after rinsing in physiological saline and draining on soft cotton cloth until the surface is barely moist;

the term *alcoholic weight* means the weight of tapeworm material after storage for one month in 95% ethanol, and subsequent drainage until superficially dry; and *dry weight* means the weight of the worm material after desiccation to constant weight, usually for 4–5 hr. at 150° C., care being taken to avoid charring. Among 100 specimens, the alcoholic weight was found to be 50.1% of the fresh weight, the dry weight was 10.1% of the fresh weight.

3. Tapeworm Longevity

Very little accurate information is available concerning the longevity of tapeworms whether *in situ* or *in vitro*. The author (18) has summarized previously such information as was available up to 1933, and can add to that summary merely the observation by Seddon (13) that in Australian lambs *Moniezia expansa* lives not longer than 65–70 days; the observation by Gordon (8) that in Australian lambs *Moniezia expansa* produces eggs for at least 350 days; and the observations of Leiper (9) of the persistence of *Diphyllobothrium latum* in man during five years and of *Diphyllobothrium mansoni* in dogs for eight years.

The difficulty in observations of tapeworm longevity *in situ* is that of eliminating the possibility of host re-infestation, a difficulty that has been stressed by Ward (16) in a critical analysis of a large number of cases of reputed longevity in *Diphyllobothrium latum*.

The difficulty in observations of tapeworm longevity *in vitro* is that of deciding whether the tapeworms are alive at all, and of deciding the exact moment of death. Absolute values of tapeworm longevity *in vitro* are of little value as physiological data since for part of the time the animal may be moribund or disintegrating. Nor can it be assumed, as was done by Cook and Sharman (6) that carbon dioxide production is indicative of tapeworm vitality, since the experiments of Brand and Weise (5) suggest that carbon dioxide production by tapeworms is a by-product of glycogenolysis and not a by-product of aerobic respiration, and it is possible that such glycogenolysis is as much a post-mortem as a pre-mortem phenomenon. In the light of Brand's results, the belief of Cook and Sharman that *Moniezia expansa* will live 200 hours in their saline solution plus *M*/10,000 sodium hydroxide needs confirmation.

The author (17, 18) has attempted to show in previous papers that tapeworms *in vitro* pass through a period of undulant activity, sometimes brief, sometimes prolonged, before passing eventually into either a condition of flaccidity or a condition of tetanic contraction, both irreversible, and has argued that the termination of longevity should be taken arbitrarily as the moment when undulant activity ceases.

A series of experiments was carried out to determine the duration of undulant activity of *Moniezia expansa* in Tyrode's solution, Brand's solution, Cook and Sharman's solution, *M*/5 sodium chloride and *M*/10 sodium chloride. These salines were used alone, and again with glucose added in amounts equivalent to $M/5$, $M/10$, $M/100$, $M/500$ and $M/1000$. One set of experiments was made with sterilized media, one set with unsterilized but freshly prepared media. Each experiment was repeated five times, five worms being used each time under the conditions of temperature given above. Observations were made at hourly intervals.

The results obtained were extremely unsatisfactory. The moment of transition from activity to passivity is difficult to determine among a number of intertwined worms and there is considerable variation between individuals. The general findings were as follows:—

1. In glucose-free media or media with $M/500$ or less of glucose, the activity period varied from 24–72 hr. in Brand's solution, 24–48 hr. in Tyrode's solution, 9–12 hr. in Cook and Sharman's solution, 9–12 hr. in $M/5$ and $M/10$ sodium chloride.
2. In media containing glucose exceeding $M/500$ in amount, the period of undulant activity was always short and varied from 9–12 hr.

In none of the media that were tested, whether sterile or non-sterile, whether glucose-free or glucose-containing, at hydrogen ion concentrations between 6.0 and 9.0, could specimens of *Moniezia expansa*—only one hour distant from life *in situ*—be relied upon to remain undulant longer than 12 hr. although occasional specimens might remain so for two or three days. The fact seems to be that no two individuals of a tapeworm species will react in the same way to the adverse conditions of an artificial environment.

Taking undulant activity as a criterion of the favorableness of an artificial medium to *Moniezia expansa*, fluid saline media are unfavorable to its life *in vitro*.

No reason for the brevity of the undulant activity period in saline media can be given. The duration of the experiment was too short for such inhibition to be caused by bacterial toxins or by the by-products of metabolism–carbon dioxide, succinic acid, lactic acid. Stoppage of activity in a sugar-free medium might be suspected to arise from glycogen exhaustion were it not for the fact that, as shown in later experiments, the glycogen content is not rapidly exhausted by starvation, and in fact is considerably increased by the presence of glucose in the medium.

4. Water Content

The author (17, 18) has discussed in previous papers the change of form induced in living tapeworms by immersion in media that are hypotonic and hypertonic in the physiological sense; how tapeworms tend to expand and become flaccid in hypotonic salines, and to become increasingly foreshortened and contracted in media whose electrolyte content is gradually increased from isotonicity.

That such morphic changes are associated with changes in water content might seem to be suggested by the high water content of tapeworms (usually between 85 and 90%), by the absence from tapeworms of exoskeletal and endoskeletal structures, and by the admitted porosity of the tapeworm surface.

Experimental confirmation of this suggestion is illustrated by Table I, which indicates the mass per original gram of living *Moniezia expansa* after immersion for six hours at 36–38° C. in various aqueous media.

TABLE I

MASS PER ORIGINAL GRAM OF LIVING *Moniezia expansa* AFTER SIX HOURS' IMMERSION AT 36–38° C.

		Arabinose	Glucose	Galactose	Levulose	Maltose	Glycine	Mucin
Water	3.3							
Tyrode × ½	2.0							
Tyrode × 1	1.004	1.16	1.15	1.04	1.04	1.15	1.87	1.28
Tyrode × 2	0.70							
Tyrode × 2½	0.88	0.53	0.71	0.38	0.67	0.50	0.52	0.56
Tyrode × 3	0.88							
Tyrode × 4	0.76							
Tyrode × 5	0.61							
Sod. glycol	0.83	0.88	0.96	0.86	0.84	0.91	0.67	—
Bile saline	1.01	0.82	0.98	0.60	0.86	0.82	0.74	1.33
	Glucose, %							
	—	0.1	0.5	1.0	2.0	5.0	10.0	20.0
Phosphate saline	0.99	1.09	0.94	0.82	0.50	0.38	0.38	

Selected whole worms were used. They were rinsed, drained on soft cotton cloth, weighed, immersed in the medium for six hours, then removed, rinsed, drained, and weighed again. The media used are named in the table.

Any change in weight induced in a tapeworm by brief immersion in a fluid must be due to water loss or water gain, since the glycogen or fat changes during six hours represent extremely minute fractions of the worm's weight. The data presented in Table I suggest that least disturbance of the initial water content is induced by Tyrode's solution, phosphate saline, and bile saline, that is to say, by those media that are isotonic with mammalian tissues. In hypotonic media such as water and half-Tyrode, there is absorption of water which may account to some extent for the flaccidity of tapeworms in such solutions. As the sodium chloride content of the medium is increased, the tapeworm shows increasing water loss until, when concentrations greater than quadruple strength are used, the tapeworm is definitely losing water and contracting visibly.

The influence of added nutrients upon water content seems to vary according to the saline base, but glucose in amounts greater than 1% seems to induce water loss.

The relatively slight disturbance of the tapeworm water content by isotonic saline media would suggest that on this criterion such media are suitable for tapeworm cultivation *in vitro*. Since, however, in such media the tapeworm is far more active and more extended than it is when *in situ*, muscular activity must to some extent be influenced by penetration of ions or molecules from the medium, a conclusion arrived at previously by the author (18), from experiments with *Nybelinia surmenicola*.

5. Polysaccharide Content

Appreciable quantities of a polysaccharide substance have been shown to occur in such gut-frequenting helminths as have been analyzed, and in the germinal membrane of the vesicular larvae of taeniid tapeworms. Reference should be made to Brand (3, 4, 5) and to Schopfer (12) for a résumé of the existing literature on the question.

Among 30 specimens of *Moniezia expansa* analyzed by Brand (4) the polysaccharide content, expressed in terms of reducing sugar to bring the figures into line with those presented below, and assuming that Brand used Pflüger's factor of 0.927, ranged from 0.99 to 5.35%, with a mean value of 2.69% of the fresh weight. This polysaccharide content may be isolated by the method of Good, Kramer and Somogyi (7) and its reducing sugar content estimated iodometrically by the method of Shaffer and Hartmann (14); or it may be further purified by the method of Somogyi (15) and dried over calcium chloride. Thus prepared from *Moniezia expansa*, the polysaccharide constituent is an amorphous white powder, readily soluble in water, beginning to char at 210° C. and attaining maximum charring at 245° C. When hydrolyzed by heating for three hours in 3% hydrochloric acid at 90° C., it yields a reducing sugar content of 95.27%; the nature of the residue was not determined. The reducing sugar obtained answers all the recognized criteria of *d*-glucose, and the polysaccharide appears therefore not significantly different from mammalian glycogen.

Among 16 selected worms, analyzed within one hour of removal from the host, the polysaccharide content expressed in terms of reducing sugar, and using the conversion factor of 0.961 suggested by Bell and Young (1), ranged from 0.35 to 5.23% of the fresh weight, with a mean value of 3.14%. Among 17 worms analyzed after one month of storage in 95% ethyl alcohol, the polysaccharide content similarly expressed ranged from 0.52 to 4.89% of the fresh weight, with a mean value of 3.31%. Among 400 thirds of *Moniezia* individuals, after one month of storage in ethyl alcohol, the polysaccharide content ranged from 1.55 to 3.96% of the fresh weight, with a mean value of 3.22%. Ethyl alcohol therefore, as could be expected, inhibits glycogenolysis and is a convenient storage fluid for material whose polysaccharide content is to be estimated. On the other hand, among 10 specimens stored for one month in 10% formalin, the polysaccharide content was only 0.19% of the fresh weight, so that formalin-preserved tapeworms are useless for glycogen determination.

The polysaccharide content of *Moniezia expansa*, therefore, is relatively high, since it constitutes approximately 3.2% of the fresh weight, or approximately 32% of the dry weight and may exceed the fat content or the protein content. Microscopical examination of sectioned *Moniezia* material stained by the glycogen demonstration method of Best (2) shows the polysaccharide content to be distributed uniformly throughout the parenchyma but to be absent from the cuticle, dermis, longitudinal and circular muscles, uterine reticulum and eggs. Its absence from the muscles of worms killed by plunging into boiling ethyl alcohol, suggests a definite correlation between polysaccharide content and muscular activity.

It is generally conceded by students of helminth physiology that the polysaccharide constituent of a parasitic helminth is synthesized from glucose withdrawn from the host fluids, similar synthesis from amino-acids being precluded by the lack of aerobic respiration, and that in the case of tapeworms such host glucose must enter through the cuticle and be synthesized into glycogen in the parenchymal cells. Ortner-Schönbach (11) suggests that the glycogen-gorged parenchyma acts as a source of glucose for the muscles, dermis and gonad-ducts where it is degraded, and as a source of sugar for the ovaries, vitellaria and gland cells where it is re-synthesized into glycogen. Admission must be made, however, that the hypothesis of glycogenesis from environmental glucose has received little experimental support. Ortner-Schönbach could detect no histological evidence of alterations in glycogen content between control specimens of the horse tapeworm *Anoplocephala* and specimens that had been kept for seven days in saline solutions and in saline-glucose solutions. Apart from the possibility that the experimental worms may not have been alive during the whole of the seven days, the present histological methods of demonstrating tissue glycogen are far from being sufficiently precise to indicate differences in glycogen concentration.

Brand (4) obtained inconclusive results by the anaerobic immersion of specimens of *Moniezia expansa* in Ringer-glucose solution. In four experiments, one worm showed a glycogen gain of 0.13% after six hours' immersion at 37.5° C. but the other three showed glycogen losses of 0.02, 0.03, and 0.18%. In the opinion of the present writer the experimental error in experiments of this nature may be as high as 1%, that is to say, two halves of the one worm may differ in glycogen content to that extent, so that Brand's results merely suggest that *no* glycogen change occurs when *Moniezia expansa* is immersed for six hours at 37.5° C. in an anaerobic solution of Ringer-glucose.

More suggestive are Brand's observations that eight specimens of *Taenia hydatigena*, from a dog that had received a high carbohydrate diet, had a glycogen content of 8.53% of the fresh weight, whereas specimens from a dog fed on an ordinary diet showed a percentage of 4.99%, but even these figures cannot be seriously considered unless it be shown that the difference, 3.39%, is greater than the maximum variation that occurs normally in glycogen content between individuals of this tapeworm species.

The data presented in Table II are based upon six-hour immersion experiments with *Moniezia expansa* in various media at temperatures of 36–38° C. In such experiments advantage may be taken of the fact that fractionated tapeworms live just as long as intact ones. Each experiment made use of five selected living worms; each was cut into approximate thirds; one set was fixed in boiling ethyl alcohol and stored in ethyl alcohol for one month before being analyzed for initial glycogen content; a second set of thirds was im-

TABLE II

INFLUENCE UPON GLYCOGEN (G) CONTENT, EXPRESSED IN MILLIGRAMS OF REDUCING SUGAR PER GRAM OF FRESH WEIGHT, OF IMMERSION OF LIVING *Moniezia* FOR SIX HOURS AT 36–38° C. IN VARIOUS MEDIA

Medium	Initial G	Final G	Medium	Initial G	Final G
Dist. water	27.32	30.42	Tyrode × 1, plus		
Tyrode × ½	29.2	28.4	Arabinose	29.8	15.3
Tyrode × 1	23.56	22.16	Glucose	30.4	55.3
Tyrode × 2	30.1	30.4	Galactose	30.0	19.0
Tyrode × 2½	35.5	36.1	Levulose	37.6	16.2
Tyrode × 3	25.84	21.25	Maltose	32.7	14.3
Tyrode × 4	31.42	25.13	Glucosamine	20.9	21.4
Tyrode × 5	38.8	20.18	Glycine	26.7	22.5
Sod. glycol.	29.7	14.7	Tyrosine	31.6	38.14
Bile saline	34.4	19.4	Mucin	40.5	18.9
Phosph. sal.	33.2	32.3	Bile 10%	20.0	17.8
Tyrode × 2½ plus			Bile saline, plus		
Arabinose	20.07	10.15	Arabinose	28.86	5.8
Glucose	31.6	39.87	Glucose	26.62	41.4
Galactose	33.5	27.5	Galactose	25.08	14.29
Levulose	29.12	27.72	Levulose	38.8	14.08
Maltose	37.00	22.5	Maltose	31.07	7.09
Glycine	30.3	11.3	Nutrient agar	30.2	5.6
Glucosamine	36.4	34.8	Glucosamine	47.16	35.7
Mucin	24.3	25.1	Mucin	36.6	37.8
Sodium glycocholate, plus			Phosphate saline plus		
Arabinose	34.8	31.0	Glucose, 0.1%	39.3	28.6
Glucose	32.68	37.48	Glucose, 0.5	21.4	52.1
Galactose	21.9	21.6	Glucose, 1.0	41.2	27.0
Levulose	36.8	11.7	Glucose, 2.0	31.5	0.93
Maltose	39.8	13.08	Glucose, 5.0	32.05	1.40
Glycine	38.36	33.8	Glucose, 10.0	41.6	6.8
Tyrosine	38.34	31.2	Glucose, 20.0	41.2	7.8
Mucin	28.06	26.0			
Cholesterol	36.06	4.06			
Lecithin	24.2	4.07			

mersed in the saline base for six hours, then alcoholized and stored; the remaining set of thirds was immersed for six hours in the saline base plus the nutrient, and then alcoholized and stored. Each experiment was repeated three times.

In the table the polysaccharide contents are expressed in terms of milligrams of reducing sugar per gram of fresh weight, allowance being made for water content changes induced by the media.

The differences between pre-experimental and post-experimental polysaccharide contents are of significance only where they exceed the maximum variation that may occur in polysaccharide content between successive thirds of a group of five worms. To arrive at this, four groups of five trisected worms were taken and each set of thirds analyzed after one month in alcohol storage. For each of the four groups, the maximum differences in polysaccharide content between sets of thirds were 2.0, 4.2, 6.0 and 10.0 milligrams of reducing sugar per gram of fresh weight.

In the table, therefore, differences of less than 10 mg. between pre-experimental and post-experimental data must be discarded as without significance.

It may be assumed, from the data in Table I, that when living specimens of *Moniezia expansa* are immersed in saline media for six hours at 36–38° C., under aerobic conditions that:

(1) There are no significant changes in polysaccharide content induced by immersion in distilled water, half Tyrode, full Tyrode, double, two and a half, triple, quadruple Tyrode, and in phosphate saline; that is to say, in media in which the longitudinal musculature of the tapeworm, as compared with the longitudinal musculature of the worm *in situ*, is relaxed;

(2) There is polysaccharide loss induced by immersion in quintuple Tyrode, in 1% sodium glycocholate solution, and in bile saline; that is to say, in those media in which contraction of the worm occurs or where it vigorously undulates. Tempting as it is to assume that the polysaccharide loss represents consumption by the functioning musculature, it is not impossible that despite its large molecule, glycogen may pass out through the body surface with the abstracted water. Glycogen can be detected in the mucus covering the freshly collected worms in quantity as high as 1.3 mg. per gm. fresh weight. The loss of glycogen in bile saline does not fit in with either conception since the water content loss in bile saline is negligible, and the undulant activity is scarcely more vigorous than when the worm is in Tyrode solution;

(3) There is a gain in polysaccharide content when glucose is present in concentrations of 1% in Tyrode's solution, bile saline, and probably two and a half Tyrode, but in sodium glycocholate and phosphate saline the polysaccharide content in the presence of 1% glucose remains unchanged;

(4) No polysaccharide gain was obtained by the substitution for glucose of other sugars, of amino acids and of glycoproteins, nor, in phosphate saline, of higher concentrations of glucose.

The glycogen changes shown above do not altogether support the view of Ortner-Schönbach (11) that the primary function of tapeworm glycogen is the nourishment of the developing genitalia, but suggest rather that it is primarily a source of fuel for the longitudinal musculature when the latter is maintaining the condition of muscular tonus which characterizes the normal worm, and that it is depleted in any artificial medium in which this tonus is maintained or in which it is exaggerated into tetanic contraction, and depleted only very slowly or scarcely at all when the musculature relaxes completely or undergoes periodically repeated phases of relaxation as during undulant activity.

6. Conclusions

If maintenance of normal longevity, water content and polysaccharide content be accepted as criteria of the favorableness of artificial media to tapeworms, and if *Moniezia* be regarded as representative of tapeworms in general, the inescapable conclusion from the data presented above is that the saline media usually employed in physiological studies are useless for the study of tapeworm physiology, and that conclusions as to the nature of tapeworm metabolism that are based upon experiments carried out in such media cannot be accepted as giving an accurate picture of the physiological processes taking place in the tapeworm that is lying in an animal gut. That is to say, the bulk of information already accumulated upon tapeworm physiology, scanty as it is, is practically worthless, and workers in this field of physiology will have to adopt a technique more akin to that of the bacteriologist than to that of the physiologist. Successful tapeworm cultivation *in vitro* may require that the animal be embedded in a semi-fluid gel after preliminary aseptic treatment. The technique of such asepsis has yet to be established, and the author is unaware of any experiments in this direction beyond his own crude and unsuccessful experiments with *Nybelinia surmenicola* (18). On the other hand, the nutritional requirements of such a tapeworm when *in vitro* may prove to be less exacting than has been supposed and it may not be necessary to duplicate the nutrient complex that surrounds the tapeworm *in situ*. Glucose seems definitely the one carbohydrate requirement of tapeworms; the amino acid requirement may be equally simple; there may be no fat requirement.

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STUDIES ON THE BIONOMICS AND CONTROL OF THE BURSATE NEMATODES OF HORSES AND SHEEP

IV. ON THE LETHAL EFFECTS OF SOME NITROGENOUS FERTILIZERS ON THE FREE-LIVING STAGES OF SCLEROSTOMES[1]

By I. W. Parnell[2]

Abstract

The effect of ten of the commoner nitrogenous fertilizers on the free-living stages of Sclerostomes, is discussed. Urea is the most lethal. Under the conditions of the experiments, which are otherwise ideal for the survival of the larvae, one part of urea to 125 parts of fresh horse feces is necessary to sterilize them. The proportions of the others tested are: Calurea, 1 : 80; powdered cyanamide, 1 : 50, with granular cyanamide slightly lower; potassium nitrate, sodium nitrate and Calnitro, 1 : 20; calcium nitrate and diammonium phosphate (dry or in medium strength solution) 1 : 17; Nitro-chalk, about 1 : 16 (but should not be used in strong solution); ammonium sulphate, 1 : 14.

When urine is not available to sterilize feces against the free-living stages of bursate nematodes, the next most economical group of chemicals to use will probably prove to be artificial fertilizers, since part, or all, of their value should be recovered in increased fertilizing value and increased crop yields.

This paper reports on the lethal values of ten of the more common nitrogenous artificial fertilizers, *viz.*,

Urea
Calurea
Calcium cyanamide
Potassium nitrate
Sodium nitrate
Calnitro
Calcium nitrate
Diammonium phosphate
Nitro-chalk
Ammonium sulphate

Tests with a few other nitrogenous chemicals will be described in subsequent papers: these will include calcium cyanide, ammonium carbonate, ammonium chloride, ammonium sulphide, ammonium nitrate and chloropicrin.

In addition to considerations of the relative cost of the fertilizers, of types of land and of crops for which the treated feces are to be used, the fact that some fertilizers or chemicals, when mixed with feces, caused loss of nitrogen as free ammonia must be remembered when a decision is being made as to which is the most economical sterilizing agent. Loss of nitrogen is most likely to occur through chemical reactions when alkaline fertilizers are mixed with manure; loss may also occur through bacterial action.

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All the fertilizers mentioned above were tested for loss of ammonia by mixing one part of the chemical with 10 parts of fresh horse feces, and incubating them under similar conditions to the other tests reported in this paper. The temperature was 25 ± 2° C. and it seems legitimate to assume that decomposition would be comparatively rapid. The fertilizers were examined for the presence of free ammonia after three and seven days. The greatest loss was found to occur with urea, Calurea, and cyanamide.

Few data are available on the chemical control of the free-living stages of parasitic nematodes, and of those many are not comparable with the present series of experiments, because the fertilizers were tested either on eggs or larvae without the presence of feces or, if tested in feces, the criterion was the absence or presence of colonies of larvae on the side of the culture jar. The first of these would not occur in practice while the second does not demonstrate the survival of a few larvae. However, the results obtained by Nöller and Schmid (7) and Enigk (2) with horse Sclerostomes, suggest that ammonium sulphate, sodium nitrate and calcium cyanamide, even in dilute solutions, do have some effect.

The work on the control of hookworm larvae with artificial fertilizers is more comparable. Large quantities of ammonium sulphate and Chili nitrate have been tested in China, at the rate of one part of fertilizer to three of night soil, and it has been shown that the larvae are quickly killed (1). In Italy, Penso (14), who has tested calcium cyanamide and other chemicals, recommends the use of calcium cyanamide for sterilizing human feces against hookworms, and also recommends (15) the addition of 12 kg. of the fertilizer to sterilize one cubic metre of manure against the Strongyles of domestic animals. Steverel and Berny (18) and de Giorgi (3), who used a 0.3% solution, agree that calcium cyanamide is lethal to Ancylostome eggs and larvae.

Newbigin and Morgan (5), in investigating chemicals for the control of the nematodes of poultry, found that 224 lb. of sulphate of ammonia per 120 sq. yd. was of no value in destroying non-bursate nematode eggs on poultry runs.

Artificial fertilizers have, however, been tested more frequently against the plant nematodes, *Anguillulina dipsaci*, *Heterodera schachtii* and *H marioni*. Unfortunately, the fertilizing effect of the chemicals on the plants has usually masked considerably the effect on the nematodes. This has made it difficult to compare the results on plant nematodes with those on Sclerostome larvae, while varied soil, climatic and other conditions tend to make the results contradictory. Nevertheless, some selected results may at least be suggestive to those interested in the control of the free-living stages of nematodes. Newton, Hastings and Bosher (6) have shown that urea, ammonium sulphate, potassium nitrate, ammonium carbonate and ammonium chloride in 2% solutions are not rapidly lethal to the bulb nematode. Hurst and Triffitt (4), found that, weight for weight, urea and calcium cyanamide were equally efficient against *H. schachtii*, but that on a nitrogenous basis, calcium cyanamide was the more effective. Small (17) failed to control

H. marioni on tomatoes with nine ounces of calcium cyanamide per square yard. Watson (20) states that good results can be obtained with half a ton of cyanamide per acre on sandy loam, and reports effective sterilization with 600–800 lb. of sodium cyanide per acre to which 900–1,200 lb. of ammonium sulphate is immediately added. Watson (19) has also shown the importance of thorough mixing of the fertilizer and the soil and, under certain conditions, of not applying cyanamide as a solution, but of immediately irrigating the land. He also finds that in soils containing a considerable proportion of colloids, three or four times as much cyanamide is necessary to control plant nematodes.

The practical possibilities of controlling bursate nematode larvae by means of artificial fertilizers in manure heaps, based partially on data reported in this paper, have already been discussed (8, 9, 13), as has the comparative inefficiency of these fertilizers against larvae on pastures (10).

It is interesting to note that the three nitrogenous fertilizers that lose most ammonia when mixed with feces are the most lethal, while pure ammonia water has been shown to be lethal (12).

The technique described in Part II of this series of papers (11), designed to allow the comparison of the lethal properties of various chemicals, has been used for all the substances reported upon here. The results immediately above, at, and below the lethal point are plotted by means of block diagrams on a logarithmic scale similar to those used in Part III (12). To confirm these results, cultures have been made with greater and smaller amounts than those illustrated, but as in every case they were in agreement with the diagrams they have not been recorded.

The conventions described in the third paragraph of Part III of this series (12) have been followed in this article also.

Because of the limited number of cultures which have been made and the numerous variable factors involved, it is impossible to state absolutely accurately at what proportion of fertilizer to fresh feces complete sterilization against the free-living Sclerostomes takes place. However, a study of the diagrams should indicate the proportion of fertilizer which may be expected to prove effective in practice, even against Sclerostome eggs on the edge of a midden, where conditions for development may be as favorable as in the cultures. It will be noticed that each fertilizer was re-tested in duplicate, not only at the quantities at which sterilization occurred in the preliminary cultures, but with smaller and greater quantities, to make certain that the results were not accidental. However, because of the numerous variables involved, the differences in the scale of quantities of fertilizer used have been left wide. Furthermore, it is hoped that when a study is made of the results obtained, both at the lethal and sub-lethal points, indications will be given on how various chemicals or groups of chemicals act on the eggs or larvae, and on the facility with which the larvae may avoid their action when buried in feces. In an earlier paper (11) it has been explained that occasionally a few larvae have been counted in a culture that should really have been negative, either because some eggs were squeezed on to the side of the container when the culture was being made and so escaped contact with the chemical, or by contamination of glassware, which could only be washed and not sterilized. Sometimes this explanation does not appear to be valid, because a few larvae occur more or less regularly in cultures treated with quantities of a chemical above the apparent lethal point. In these cases it may be an indication that that chemical is very local in its action—a most important point in practice—and is not capable of reaching eggs or larvae that remain inside a lump of feces. Many of the gas-producing chemicals have this peculiarity. This difficulty should be largely overcome by using chemicals that are soluble and highly lethal in weak solutions, to allow thorough wetting of all feces. Alternatively, the survival of a few larvae may be due to the extraordinary resistance of the eggs or larvae of one or more of the numerous species which occur in horses.

When only a few larvae in a culture survive, it seems to be a point of legitimate speculation whether, if they had survived under similar conditions in nature, they would be able to survive the hazards of life in the host to maturity. While it has been absolutely impossible to attempt any infection experiments with these larvae, it has been possible to keep some of these cultures for a few months in the laboratory; in some cases, their life does not appear to have been as long as that of the controls.

Urea

Fig. 1 shows the effects of urea on the numbers and condition of the infective Sclerostome larvae obtained from 40 gm. of horse feces treated with the fertilizer within four hours of being passed. Urea ($CO(NH_2)_2$) theoretically contains 46.6% nitrogen (16).

Urea was tested dry in quantities ranging from ⅛ of 1% to 20% of the feces, by weight. Sterilization against Sclerostomes was effective in any culture which was treated with 0.33 gm. or more; *i.e.*, more than 0.83% of dry urea is lethal. Quantities slightly less than this, however, were partially effective. In solution, urea is equally potent. With a 1 : 2 aqueous solution, effective sterilization is produced by 0.75 cc. or more, containing 0.28 gm. of urea; this is equivalent to 0.7% of urea to feces by weight. With a 1 : 4 solution, 1.5 cc. effects sterilization; this quantity contains 0.32 gm. of urea, or 0.8% of the weight of the feces. The results obtained with a 1 : 8 solution were less regular, but in practice it will probably be found that, at the equivalent rate of slightly over 2.0 cc. to 40 gm., effective sterilization will be effected; 2.0 cc. contains only 0.23 gm. of urea. Five cc. and 7.5 cc. of a 1 : 20 solution produced sterilization in two of the three cultures treated with both quantities, while with 10.0 cc. and more the results were uniformly effective; 7.5 cc. of a 1 : 20 solution contains 0.36 gm. of urea, equivalent to 0.9% of the weight of the feces. Since urea was so lethal, it was also tested as a 1 : 50 solution. At this strength 20.0 cc. were necessary and 15.0 cc. caused a considerable mortality. Twenty cubic centimetres of this solution contain about 0.39 gm. of urea or 0.99% by weight.

When 20.0 cc. of fluid, or even less, is added to a 40 gm. culture, there tends to be some drainage from the culture, and, therefore, some of the fertilizer may not act on the eggs or larvae. Accordingly, never more than 25.0 cc. of fluid was added.

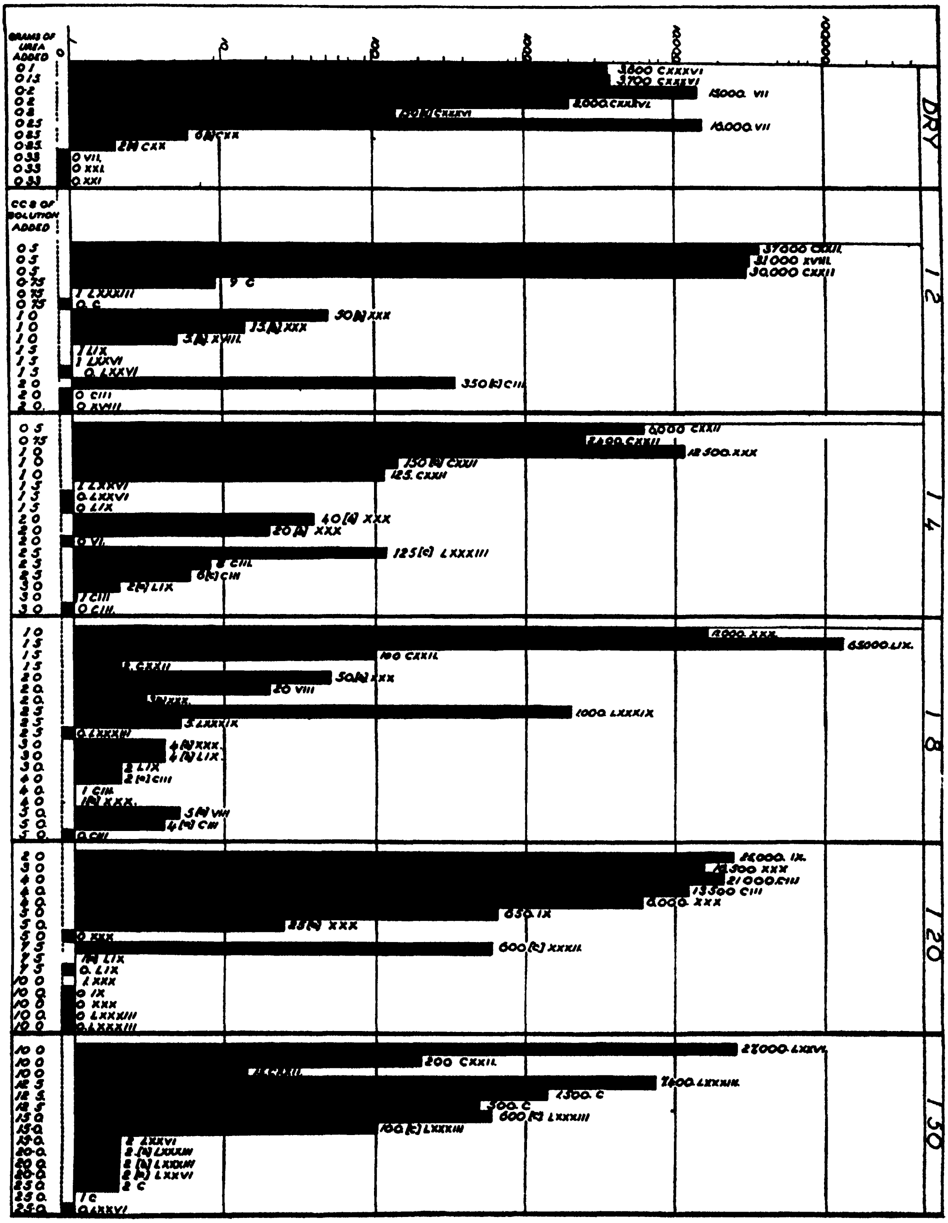

FIG. 1. *Results of tests with urea, dry and in solution. Roman numerals refer to the controls shown in Table I. a, after a number means that all, or practically all, the larvae were dead; b, that a considerable proportion were dead; c, that a few were dead. The letters have the same significance in subsequent figures.*

These figures suggest that one third of a gram of urea to 40 gm. of feces (or 0.83%) whether dry or in solution, will prove to be effective as a sterilizing agent against Sclerostomes in fresh feces.

Calurea

Fig. 2 shows the results obtained with Calurea. This fertilizer, containing 34% nitrogen, is a double salt of calcium nitrate and urea (16).

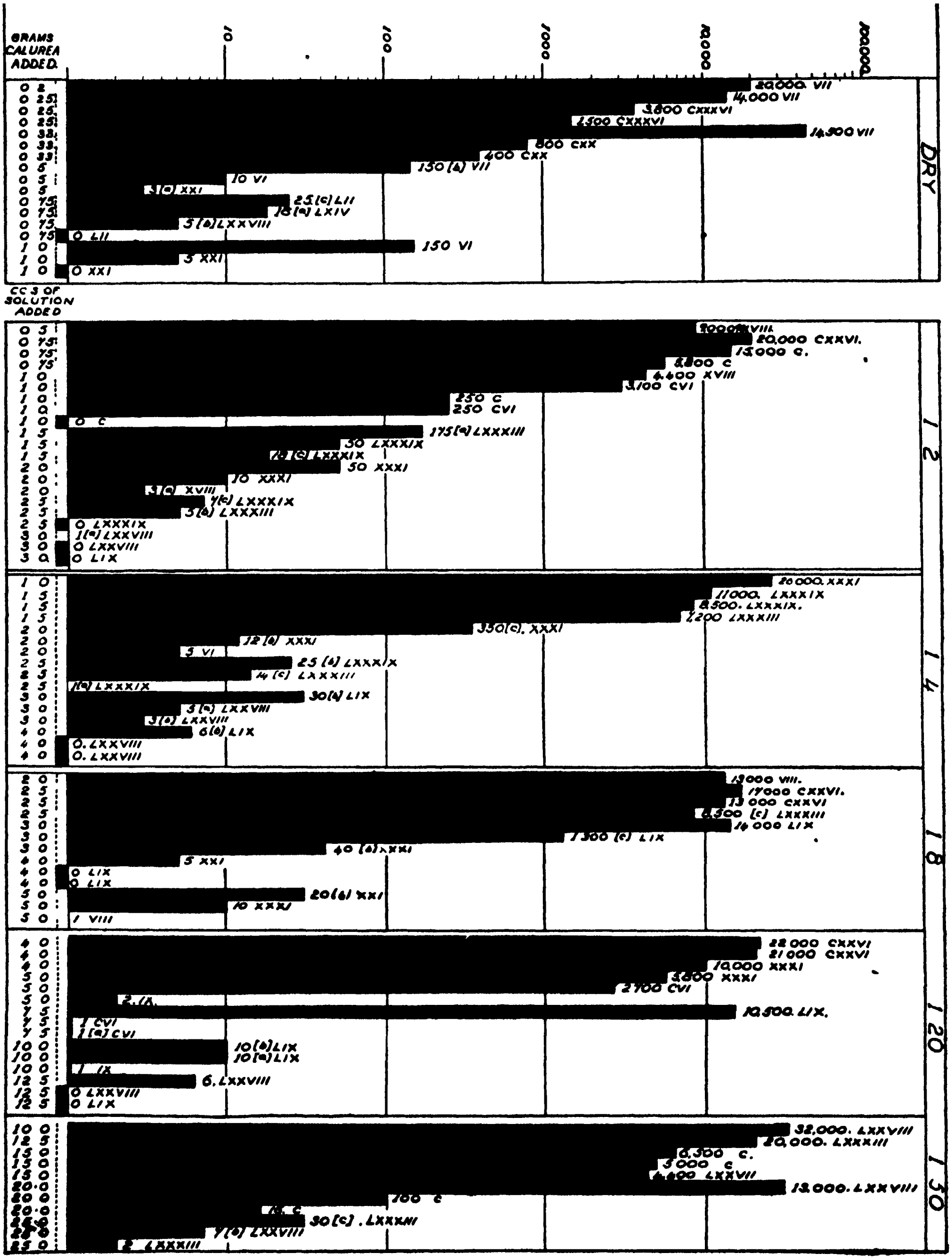

FIG. 2. *Results of tests with Calurea, dry and in solution.*

Calurea, dry, was tested in proportions of ¼ of 1% to 20% of the weight of fresh feces. The results are somewhat irregular, but suggest that 0.5 gm. in 40 gm. (or 1.25%) of Calurea would be effective. With solutions the results are also rather irregular; 1.5 cc. of a 1 : 2 solution, containing 0.57 gm., and 2.0 cc. of a 1 : 4 solution, containing 0.44 gm., approximate to the minimum quantities necessary to effect sterilization. This is equivalent to 1.33% and 1.1% of Calurea to feces. With a solution of one part in eight of water, 4.0 cc. produced sterilization; this quantity contains 0.47 gm. or 1.17% of Calurea to feces. Ten cc. or slightly less, of a 1 : 20 solution and 25.0 cc. of a 1 : 50 solution were effective; both these quantities contain 0.49 gm., equivalent to 1.23% of Calurea to feces. The results obtained with these cultures suggest that 0.5 gm. to 40 gm. of feces or 1.25% of Calurea would be effective in fresh feces.

Calcium Cyanamide

Fig. 3 illustrates the results obtained with dry powdered and granular cyanamide. Fig. 4 illustrates the results with powdered cyanamide in "solution". Calcium cyanamide ($CaCN_2$) is marketed in two forms—"pulverized", "powdered" or "oiled", and "granular"; the latter form is made by the use of nitrate of lime as a binding material, and obviates the difficulties of handling, which have been a disadvantage of the pulverized form as an artificial fertilizer. Since this substance may be toxic to plants, especially seedlings, during the early break-down processes, freshly treated manure should not be applied to land immediately before seeding. The usual reaction in soil is for this fertilizer to be transformed to urea and then rather slowly to nitrate. Moisture hastens the process. The sample of powdered cyanamide

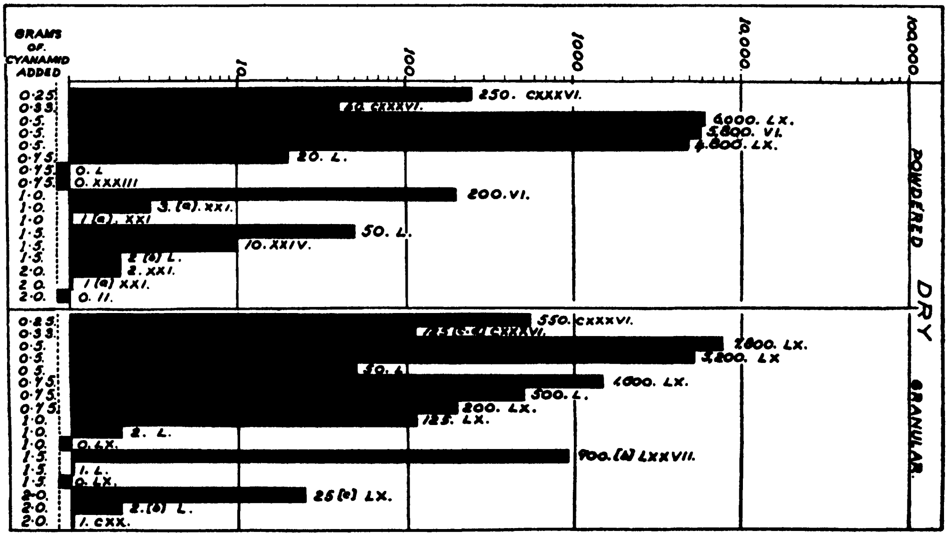

FIG. 3. *Results of tests with dry powdered, and granular cyanamide.*

which was used in these cultures contained 22% nitrogen and 70% calcium hydroxide; the granular form contained 21% nitrogen. (The results obtained with various compounds of calcium, which are comparatively ineffective, will be included in the paper on non-nitrogenous fertilizers.) Granular cyanamide was not tested in solution, since if a "solution" is required it is obviously

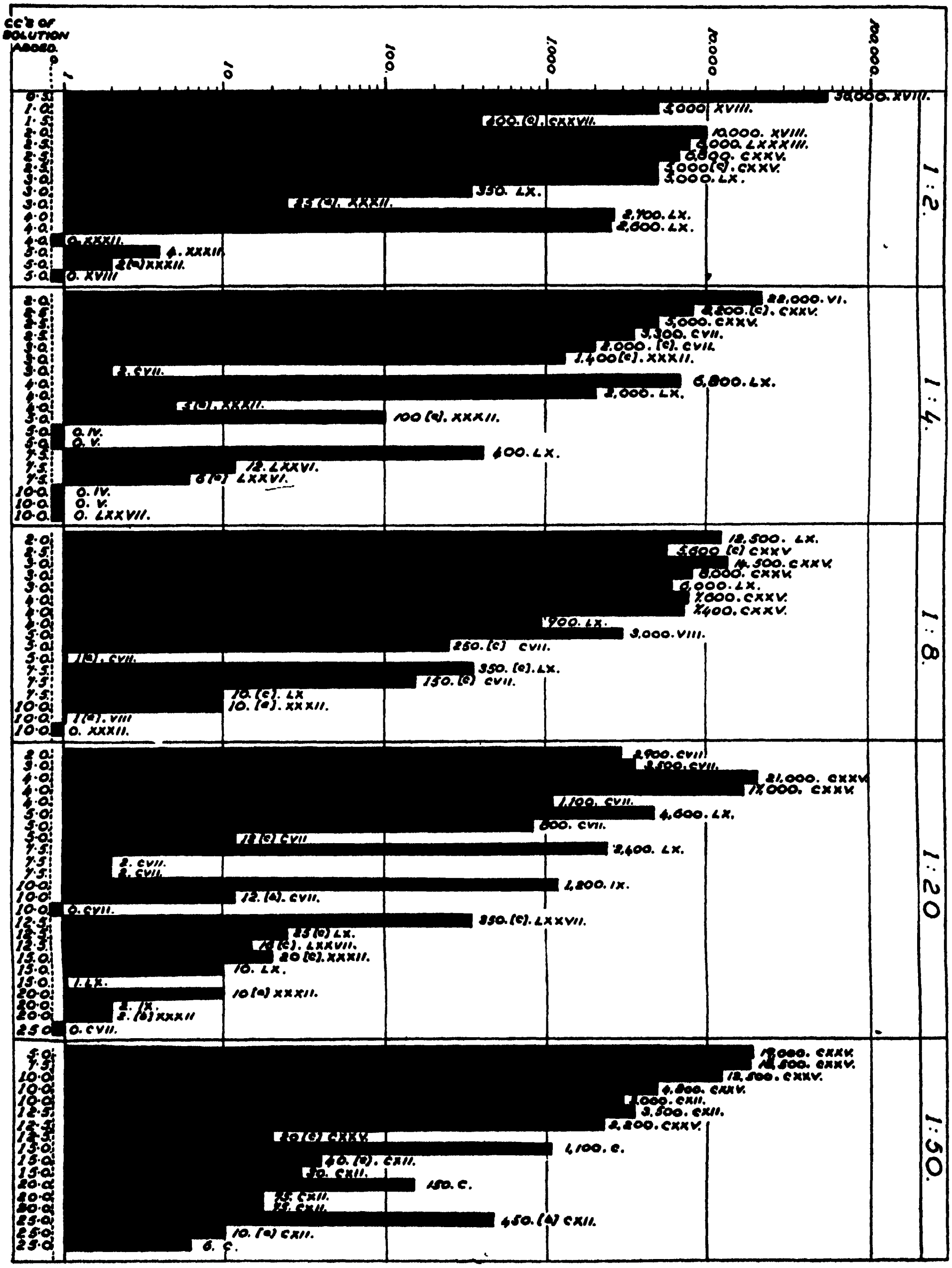

FIG. 4. *Results of tests with powdered cyanamide in "solution".*

unnecessary to use the granular form. However, 1 : 2 and 1 : 4 "solutions" of powdered cyanamide were tested, in spite of the fact that they have the consistency of mud, and would be quite useless in practice owing to the impossibility of thoroughly mixing with feces.

The results obtained with granular cyanamide were rather more irregular than those produced by the powdered form, but indicate that 0.75 gm. of dry powdered cyanamide and 1.0 gm. of the granular form are approximately the quantities necessary to sterilize a 40-gm. culture, equivalent to 1.9% and 2.5% of fertilizer to feces.

Probably owing to the difficulty of efficient mixing, 5.0 cc. of a 1 : 2 solution and the same quantity of a 1 : 4 solution had to be mixed with 40 gm. of fresh feces to kill most of the Sclerostomes; 5.0 cc. of these two solutions contain 1.9 and 1.1 gm., equivalent to 4.75% and 2.75%. Ten cc. of a 1 : 8 cyanamide-water solution was effective, while 7.5 cc. or even 5.0 cc. caused a high mortality; 10.0 cc. contain 1.2 gm., equivalent to 3.0%. The interpretation of the results obtained with 1 : 20 and 1 : 50 solutions is also difficult. It can be inferred, however, that smaller amounts of cyanamide are required with these weaker "solutions." It appears that 15 cc. is the quantity of 1 : 20 solution required to cause almost complete sterilization with regularity. This quantity contains 0.75 gm. At least 25 cc. of 1 : 50 solution is necessary for sterilization; this quantity contains slightly less than half of one gram of cyanamide, equivalent to only 1.25%.

Potassium Nitrate

Potassium nitrate (KNO_3), or saltpetre, in the pure form contains less than 14% of nitrogen. Other potash salts will be discussed in the paper dealing with non-nitrogenous artificial fertilizers. Fig. 5 shows the results obtained with potassium nitrate. Dry, this fertilizer was irregular in its action, requiring about 3.0 gm. to effect sterilization, although 2.0 gm. almost sterilized the cultures. In solution, 5.0 cc. of a 1 : 2 solution, containing 2.0 gm., is the minimum quantity at which sterilization can be expected, although smaller amounts were almost effective in a few cases, and with 7.5 cc. some larvae survived. A 1 : 4 solution was almost effective with 7.5 cc.; this contains 1.75 gm., equivalent to 4.4% of fertilizer to feces. The results with a 1 : 8 solution also were irregular, the three cultures treated with 10.0 cc. being virtually sterilized, but some larvae survived in the cultures treated with 12.5 cc.; 15 cc. of a 1 : 8 solution contains about 1.8 gm., or 4.5%. Sterilization did not occur in the cultures treated with a 1 : 20 solution. These results suggest that about 5% by weight of potassium nitrate must be added to fresh feces to kill the free-living Sclerostomes.

Sodium Nitrate

Sodium nitrate ($NaNO_3$), or Chili saltpetre, contains 15% to 16% of nitrogen, as nitrate, and therefore, is a very quick-acting fertilizer.

In addition to the cultures tabulated in Fig. 6 which show the results obtained with sodium nitrate, some cultures in which straw was incorporated, were made with this fertilizer, and with ammonium sulphate. In both cases, the addition of straw considerably increased the proportion of fertilizer necessary.

Dry, 2.0 gm. of sodium nitrate, or 5% of the weight of feces is probably approximately the necessary proportion to obtain sterilization of fresh feces (although numerous larvae survived in one culture treated with this quantity). The quantities of 1 : 2, 1 : 4 and 1 : 8 solutions necessary to produce sterilization approximate to 7.5 cc., 10.0 cc. and 12.5 cc. containing 3.1 gm., 2.3 gm.

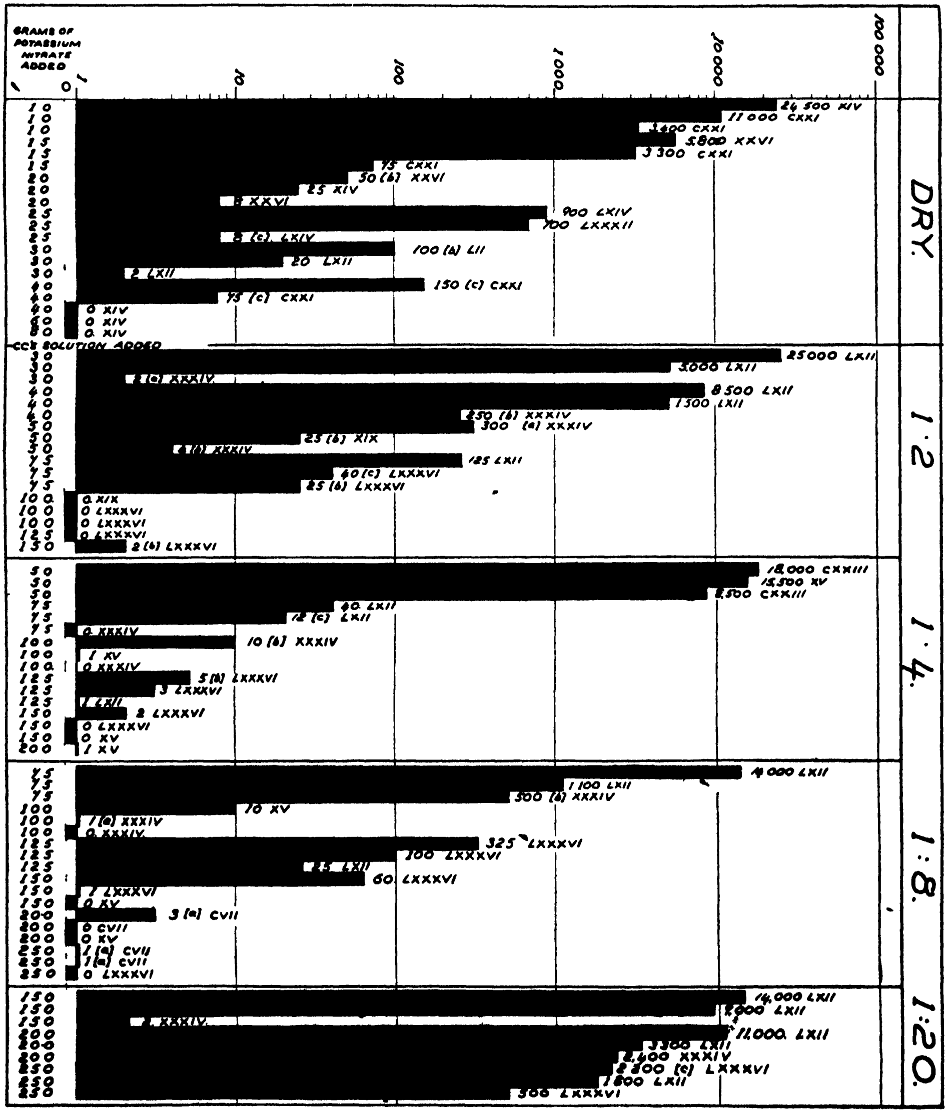

FIG. 5. *Results of tests with potassium nitrate, dry and in solution.*

and 1.5 gm. equivalent to 7.75%, 5.75% and 3.75%. However, in each case a few cultures were practically sterilized with lesser amounts. The larger quantities of a 1 : 20 solution greatly reduced the number of surviving larvae and almost effected sterilization. These cultures indicate that dry, or as a medium-strength solution, sodium nitrate is effective when used at the rate of about 5% by weight of the fresh feces with which it is mixed.

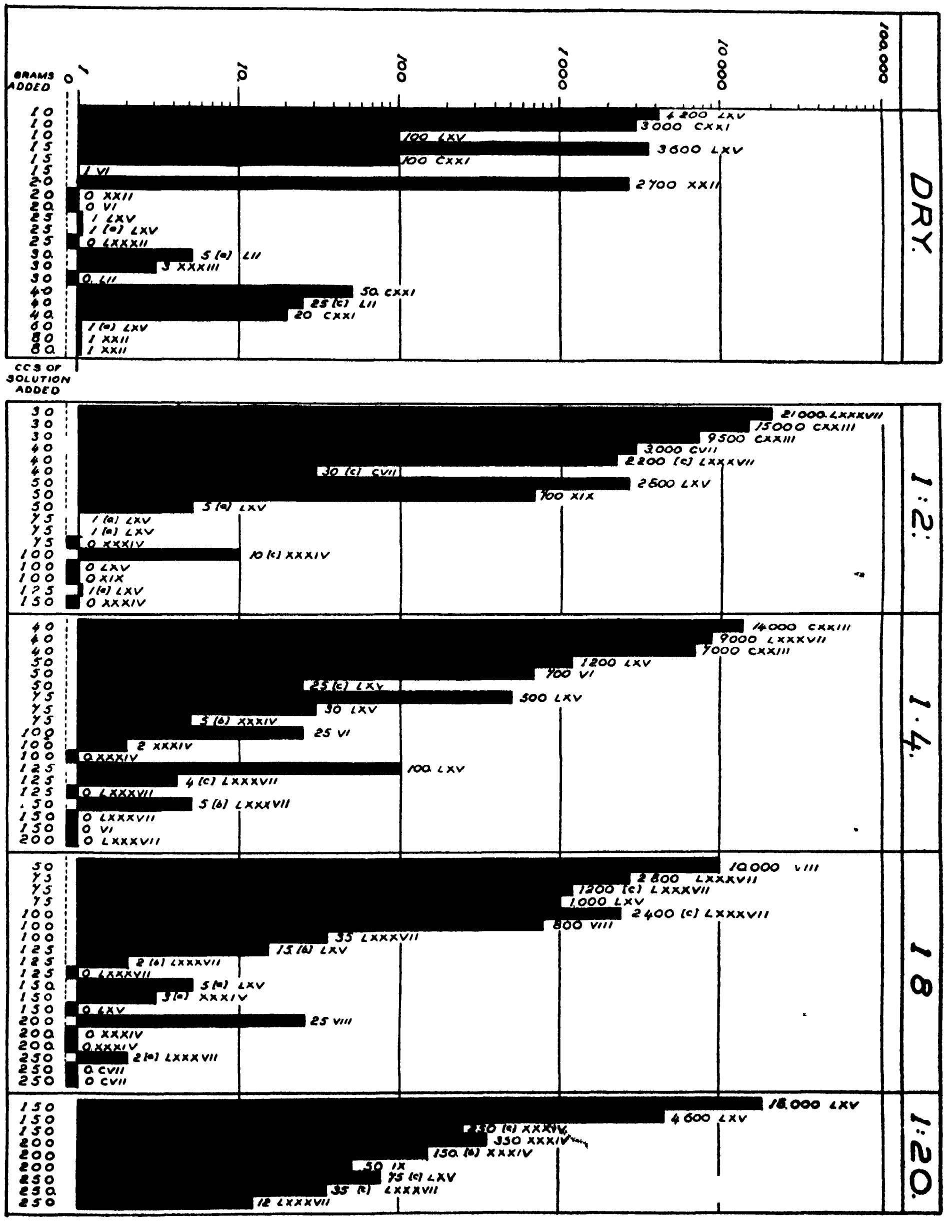

FIG. 6. *Results of tests with sodium nitrate, dry and in solution.*

Calnitro

Fig. 7 shows the results obtained with Calnitro, a fertilizer made by treating ammonium nitrate with calcium carbonate. It contains about 20% of nitrogen.

Mixed dry with feces, Calnitro produced somewhat variable results, but when the two cultures which were sterilized by 1.5 gm. are balanced against the one which was not sterilized by 2.0 gm., the assumption may be made that the Sclerostomes are killed when about 5% of fertilizer is mixed with the fresh feces. As a 1 : 2 solution, Calnitro was effective when a quantity of 7.5 cc. was used; this contains 3.0 gm. or 7.5%. As a 1 : 4 solution,

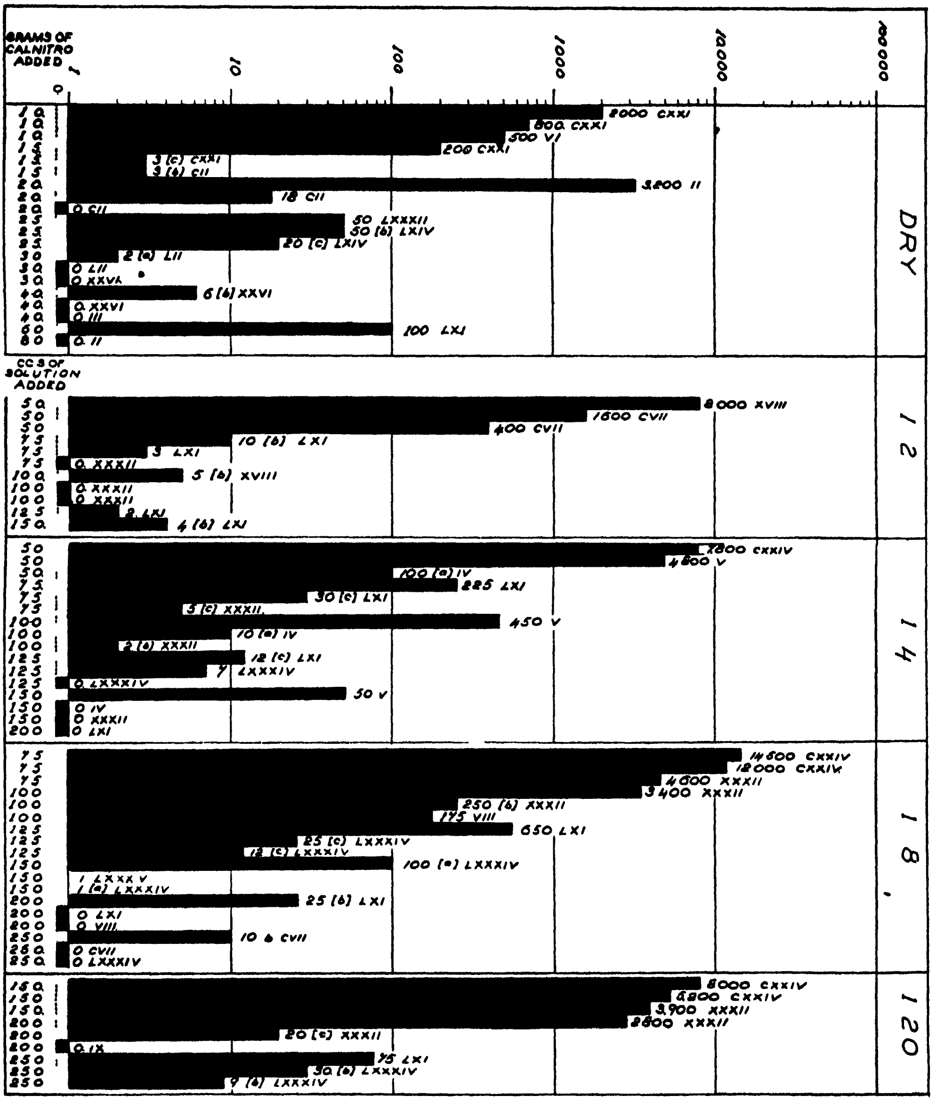

FIG. 7. *Results of tests with Calnitro, dry and in solution.*

Calnitro was more effective, but the exact value of its sterilizing effect was more difficult to interpret. Probably it approximates to 10.0 cc. containing 2.25 gm., equal to 5.6% of Calnitro to feces. Fifteen cubic centimetres of a 1 : 8 solution was effective, although less caused a considerable mortality. This quantity of solution contains 1.8 gm., or 4.5%. The quantity of a 1 : 20 solution required to sterilize 40 gm. of fresh feces is probably slightly less than 25.0 cc.; this quantity contains 1.25 gm. or the equivalent of only 3.1%.

Calcium Nitrate

Fig. 8 illustrates the sterilizing properties of calcium nitrate ($Ca(NO_3)_2$) against Sclerostome eggs or larvae when mixed with fresh feces. This fertilizer contains about 16% nitrogen.

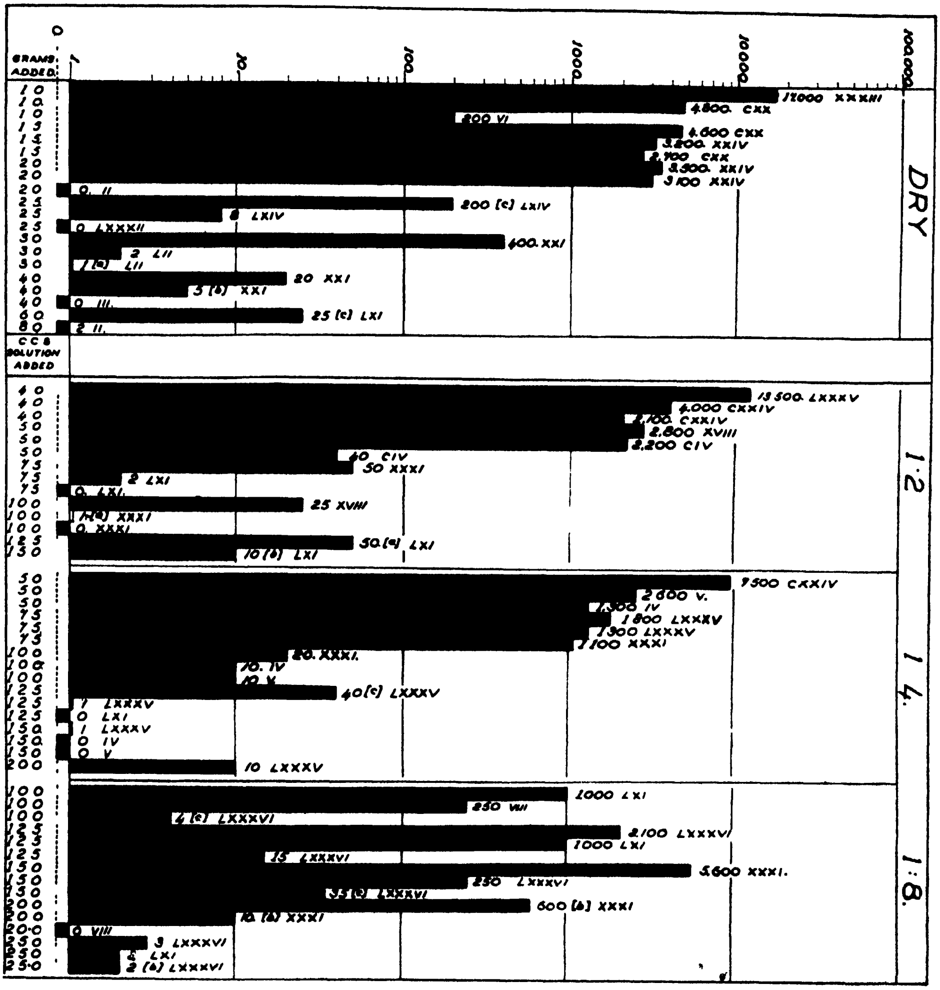

FIG. 8. *Results of tests with calcium nitrate, dry and in solution.*

Used dry, calcium nitrate is more or less effective at the rate of 2.5 gm. to 40 gm. of feces, or 6.25%. As a 1 : 2 solution, 7.5 cc. is required to obtain sterilization; this quantity contains 3.0 gm. of fertilizer or 7.5% of fertilizer to feces. Ten cubic centimetres of a 1 : 4 solution, containing 2.25 gm. is effective; this is equivalent to 5.66% of calcium nitrate to feces. As a 1 : 8 solution, the results are more irregular, 10.0 cc. sterilizing one culture, but 20.0 cc. or even 25.0 cc. being necessary for reliable results. Twenty cubic centimetres contain 2.4 gm., which is 6%. Twenty-five cubic centimetres of a 1 : 20 solution considerably reduced the number of larvae but did not produce effective sterilization. Dry, or as a medium-strength solution, calcium nitrate must, therefore, be used at the rate of about 6% of the weight of feces to kill Sclerostomes, under conditions which are probably not only ideal for the development of the larvae, but for the fertilizer to exert its lethal action.

Diammonium Phosphate

Diammonium phosphate ($(NH_4)_2HPO_4$) contains nearly 20% nitrogen and 53% phosphoric acid. Fig. 9 shows the results obtained with this fertilizer. Other phosphatic fertilizers will be considered in the paper dealing with non-nitrogenous fertilizers.

Dry, 2.5 gm. of diammonium phosphate effectively sterilized 40 gm. of fresh feces, while a few cultures were sterilized with a smaller quantity. As a 1 : 2 solution, the results were most irregular. Two cultures were completely sterilized by 7.5 cc. or 3.0 gm., but in the third culture many larvae survived; 10.0 cc. was effective. The 1 : 4 and 1 : 8 solutions also produced highly irregular results. In many cultures larvae reached the infective stage and died shortly afterwards.

Allowing for the difficulty in interpreting these results, it is probable that 10.0 cc. of a 1 : 4 solution, containing 2.2 gm. or 15.0 cc. of a 1 : 8 solution containing 1.8 gm., equivalent to 5.5% and 4.5% of fertilizer to feces, are the amounts required to obtain sterilization. With a 1 : 20 solution, only reduction of numbers and not complete destruction of the larvae was effected.

These results suggest that about 6% of diammonium phosphate to fresh feces is necessary to sterilize the latter against free-living Sclerostomes.

Nitro-chalk

Nitro-chalk also is a product made with ammonium nitrate, with finely divided chalk. Half the nitrogen, which forms 15.5% of the fertilizer, is present as ammonia and half as a nitrate. In Fig. 10 the sterilizing action of this fertilizer against free-living Sclerostomes in fresh feces is shown.

The action of dry Nitro-chalk is somewhat irregular, but the ratio required to effect sterilization appears to be about 3 : 40. As a 1 : 2 solution, the quantity necessary appears to be between 7.5 cc. and 10.0 cc.; the latter quantity contains 4.0 gm. A 1 : 4 solution is proportionately more effective, 12.5 cc. producing sterilization and 7.5 cc. and 10.0 cc. heavy death rates; 12.5 cc. contains 2.8 gm., or 7%. Fifteen cubic centimetres of a 1 : 8 solution, which contains only 1.8 gm., was sufficient to produce effective results; this figure is equivalent to 4.5%. The largest quantities used of a 1 : 20 solution only reduced the number of Sclerostome larvae. These results

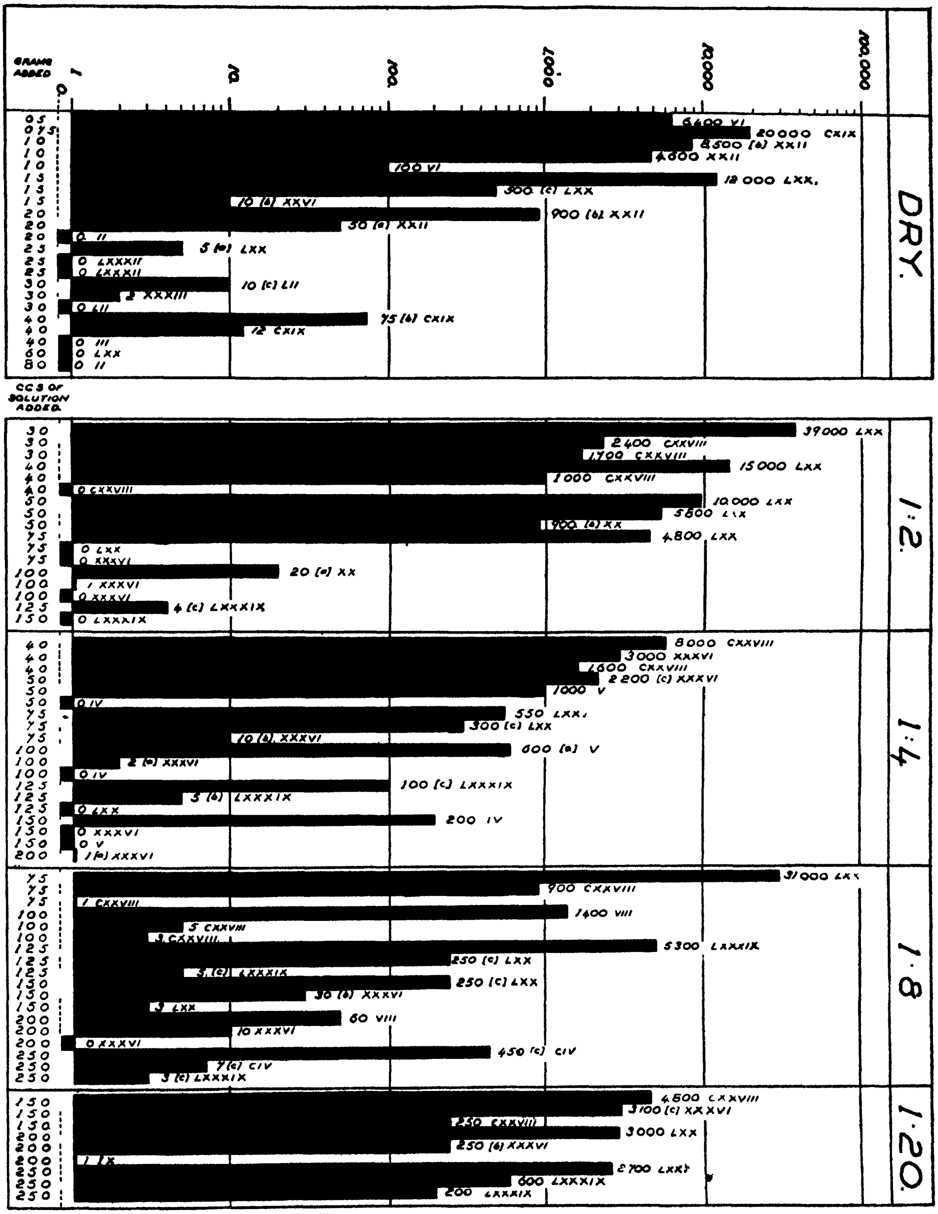

FIG. 9. *Results of tests with diammonium phosphate, dry and in solution.*

suggest that Nitro-chalk should not be used as strong or very weak solutions, and that even under optimum conditions, it is among the least lethal of the nitrogenous fertilizers.

Ammonium Sulphate

Ammonium sulphate ($(NH_4)_2SO_4$), one of the original artificial nitrogenous fertilizers, contains about 20% of nitrogen. The results obtained with this fertilizer are shown in Fig. 11.

Dry ammonium sulphate at the rate of 7.5% may be expected to produce sterilization. In solution, 7.5 cc. of a 1 : 2 solution containing 3.0 gm., and 12.5 cc. of a 1 : 4 solution—equivalent to 2.8 gm.—appear to be the minimum quantities at which sterilization regularly occurs (equivalent to

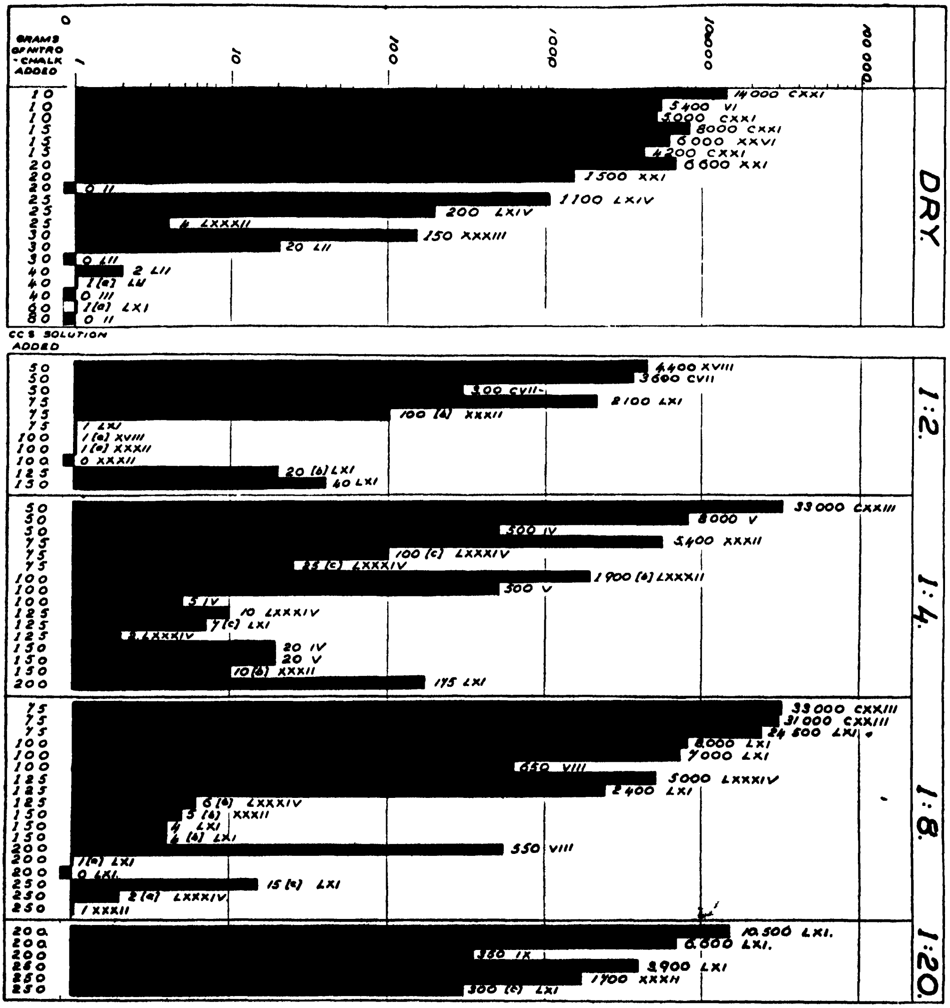

FIG. 10. *Results of tests with Nitro-chalk, dry and in solution.*

7.5% and 7%) although some cultures were sterilized with smaller amounts. The results with a 1 : 8 solution were also most irregular, but 20.0 cc. containing 2.4 gm. (6.0%) appears to be the least amount upon which reliance may be placed. Even 25.0 cc. of a 1 : 20 solution failed to reduce Sclerostome larvae to numbers small enough to have no importance in practice.

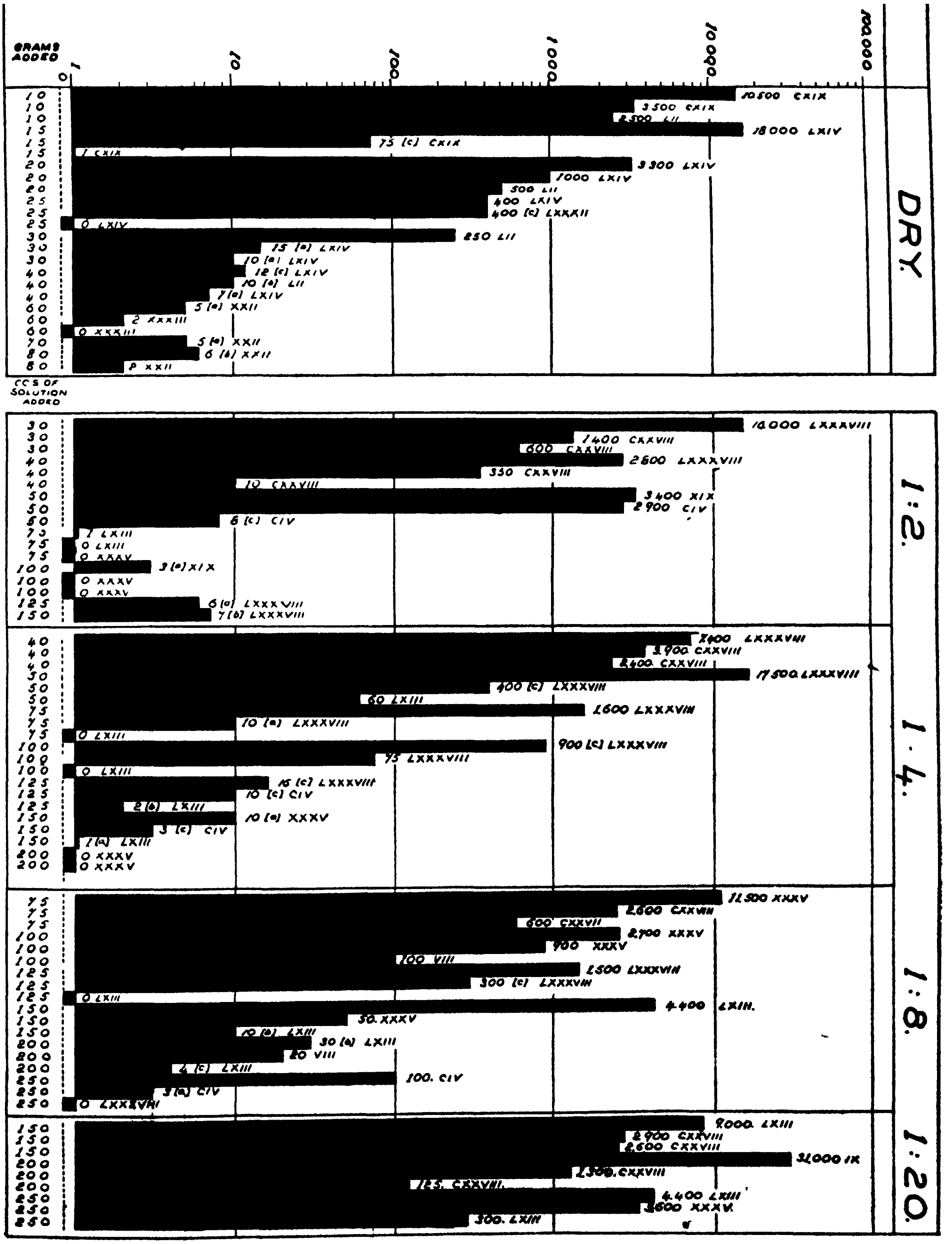

FIG. 11. *Results of tests with ammonium sulphate, dry and in solution.*

TABLE I

KEY TO FIGS 1–10

Series number	Date cultures made	Days kept in C.T. room	Average number of larvae isolated
II	August 27, 1934	10	23,000
III	August 27	17	25,000
IV	September 19	11	1,100
V	October 5	10	7,600
VI	December 12	19	21,000
VII	January 4, 1935	11	21,000
VIII	January 4	24	21,000
IX	February 13	31	44,000
XIV	May 21	23	29,000
XV	May 21	36	18,000
XVIII	May 31	32	60,000
XIX	May 31	32	13,000
XX	May 31	33	12,000
XXI	June 5	39	24,000
XXII	June 5	39	25,000
XXIV	July 9	18	72,000
XXVI	July 9	18	48,000
XXX	July 12	21	27,500
XXXI	July 12	77	35,000
XXXII	July 15	77	15,000
XXXIII	July 19	14	24,000
XXXIV	July 22	82	32,000
XXXV	July 22	84	23,000
XXXVI	July 23	92	23,000
L	November 21	28	25,000
LII	November 22	25	62,000
LIX	December 17	28	32,000
LX	December 23	17	45,000
LXI	December 27	21	42,000
LXII	December 27	27	16,000
LXIII	January 2, 1936	27	27,500
LXIV	January 2	29	36,000
LXV	January 2	32	11,500
LXX	January 16	19	64,000
LXXVI	January 30	20	5,000
LXXVII	January 30	22	33,000
LXXVIII	January 30	31	30,000
LXXXII	February 18	22	16,500
LXXXIII	March 3	13	25,000
LXXXIV	March 3	15	12,500
LXXXV	March 4	14	31,000
LXXXVI	March 4	19	33,000
LXXXVII	March 4	20	38,000
LXXXVIII	March 5	22	15,000
LXXXIX	March 5	25	26,500
C	March 26	21	3,600
CII	April 1	20	27,500
CIII	April 1	22	26,000
CIV	April 7	23	21,500
CVI	April 16	15	13,000
CVII	April 16	19	17,000
CXII	April 24	24	7,800
CXIX	May 15	27	59,000
CXX	May 15	29	24,000
CXXI	May 15	32	42,000
CXXII	May 22	17	4,400
CXXIII	May 26	21	49,000
CXXIV	May 26	23	40,000
CXXV	May 26	25	18,500
CXXVI	May 26	28	26,500
CXXVII	June 9	16	11,000
CXXVIII	June 9	18	10,000
CXXXVI	July 2	12	2,200

Conclusion

The proportion of nitrogenous fertilizers to feces shown in this paper as necessary to effect sterilization would, in general farming practice, be too high to be used, if all the feces had to be treated, but in a well-built manure heap, only that manure which was put on the bottom, top or sides would have to be treated, because Sclerostome larvae are unable to survive the heat of fermentation, and probably the lack of oxygen and harmful products of decomposition, in the centre of a manure heap.

Acknowledgments

It is a pleasure not only to be able to record continued help from the firms mentioned in Part II of these papers (11), but to be able to thank Canada Packers, Limited, The Chemical and Metallurgical Corporation Limited, Tobacco By-products and Chemical Corporation Inc. (U.S.A.) and British Alkaloids Limited (England) most sincerely for their generous help.

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A PRELIMINARY ACCOUNT OF THE UNIONIDAE OF ONTARIO[1]

BY A. LA ROCQUE[2] AND J. OUGHTON[3]

Abstract

This study is based on an examination of museum specimens and records in the literature. The Unionidae of Ontario comprise 45 species and 18 varieties. Their distribution in the various drainages is shown in a table and the fauna of each area is discussed. The doubtful species and three erroneous records are noted.

Introduction

As an introduction to studies of the distribution of the Unionidae of Ontario, lists that have been published for the Toronto, Hamilton and Ottawa areas and data from more comprehensive works have been brought together, and the specimens on which the lists were based have been examined wherever possible. The taxonomy has been brought up to date (under the direction of Dr. Henry Vander Schalie) and an attempt has been made to indicate briefly the known distribution. Although a large proportion of the shells collected in Ontario have been examined, some species still remain in the "doubtful" list.

The freshwater mussels and clams of Ontario can be separated into two families, the Unionidae (pearly mussels) and Sphaeriidae (fingernail clams). They may be briefly described thus:

Family Unionidae. Length, 1 to 8 in. Shape, varied; some species almost circular, others oval, pendant-like or elongate; some have grooves or knobs on the surface. Color, externally usually brown, green, straw or yellow; sometimes with clearly marked radiating lines; internally white, bluish, silver or rose; often with iridescent hues.

Family Sphaeriidae. Length less than 1 in. Shape, oval or oblong, chiefly, with no conspicuous ornaments. Color, externally straw or light brown; internally white or bluish white.

This account deals with the Unionidae only.

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[1] *Original manuscript received April 24, 1937.*
Contribution from the Royal Ontario Museum of Zoology, Toronto, and the National Museum of Canada, Ottawa, Canada.

[2] *Museum Assistant, Geological Survey, Ottawa.*

[3] *Research Associate, Royal Ontario Museum of Zoology, Toronto.*

Sources of Material

The specimens which have been examined are in the following museums:

1. University Museums (including the Bryant Walker Collection, University of Michigan).
2. The National Museum of Canada.
3. Royal Ontario Museum of Zoology.

These, together with the literature listed below, form the actual basis for this list.

The collection at Ottawa has been partly determined by Simpson and partly by Bryant Walker. Representatives of species in the Toronto collection have been examined by Dr. Vander Schalie.

These collections contain moderately extensive series of shells from the settled parts of Ontario but only a sparse representation from that vast area of the province that is west and north of the Georgian Bay drainage.

We are unable to divide certain genera represented in Ontario into well-marked species. Such "difficult" groups are *Anodonta* and *Lampsilis*. We have not been able to consider their distribution in detail. Likewise *Obovaria* and *Pleurobema* are "difficult" but of less importance since they are not common mussels throughout the province.

The size and shape of the shell in the Unionidae have been shown by Ortmann, Grier and others to be correlated with environmental differences. For example, the shell of a mussel living in a large lake may be dwarfed when compared with that of the same species dwelling in a large slow river. Specimens collected by us in the Niagara River about a mile or two upstream from the Falls, where the current is moderately fast, show similar dwarfing to that induced by lake conditions. We have listed several but not all of these "environmental varieties".

It should be noted that *Amblema costata* (Raf.) and *Amblema plicata* (Say) are herein noted separately, although it seems to us that they are not specifically distinguishable.

We concur in the opinion of Latchford who was the first to point out that *Lampsilis borealis* (A. F. Gray) (7) is no more than a variety of *Lampsilis radiata*, and have treated it accordingly herein. It is interesting to note that the original figures of this "species" were drawn by Justice Latchford and that the initials A. G. on the plate refer to Mr. A. Grignard, the lithographer, and not to A. F. Gray who drew up the description.

A Comparison of Distribution in the Various Ontario Drainages

For this purpose, we have included only 40 species and 14 varieties, having eliminated certain closely related species. The species and varieties of *Anodonta* have been considered as but one species since we feel that many of the species recorded are not valid.

I. *Hudson and James Bay Drainage*

The Hudson and James Bay drainage has a moderately large Unionid fauna but most of the species have been recorded only for Manitoba. This fauna seems to extend only slightly into Ontario. *Lasmigona complanata* may be taken as an example. In Ontario this species is definitely known only from the extreme western portion—Lake of the Woods (Dawson (4)), and Winnipeg River (Latchford (11), Mozley (13)). In Ontario waters of the Hudson and James Bay drainage in general there is apparently a very small mussel fauna, namely eight species and two varieties.

II. *Lake Superior and Drainage*

There are records for only eight species and two varieties—a list equal to that of the above drainage.

III. *Lake Huron and Drainage*

There are 24 species and eight varieties definitely known from this section. It is curious to note that, while *Elliptio complanatus* (Dillw.) is generally common in the Lake Huron drainage, it is apparently very rare in one part, Lake Simcoe. Dr. E. M. Walker, who has for a long time been acquainted with this area, has never found it in the lake. But Rawson (17) has found specimens in the mouths of creeks draining into Lake Simcoe.

IV. *Lake St. Clair, Detroit River, Lake Erie and their Drainages*

These, the most southern waters of the province, contain the greatest variety of mussel fauna, having 34, 26 and 34 species, respectively. Seven species on our list are entirely confined to these drainage areas. *Elliptio complanatus* (Dillwyn) is either absent or very rare here. Ortmann in his Monograph (15, p. 110) says concerning this species: "It has never been found in Lake Erie . . . ."

V. *Niagara River and Tributaries*

The fauna comprises 26 species and four varieties.

VI. *Lake Ontario and Drainage*

We have records for 17 species and four varieties. Two of these species, namely *Leptodea fragilis* and *Proptera alata* are very rare in Lake Ontario. They have been found only in the western end of the lake. It is possible that these two species have migrated there by way of the Welland Canal.

VII. *St. Lawrence (in Ontario) and Ottawa Rivers and Tributaries*

In this drainage section, there are 18 species and five varieties known. Part of this fauna shows affinities with that of the Atlantic slope drainage. For example, *Alasmidonta undulata* is confined, in Ontario, to this drainage section. It is found in the Ottawa River even into the headwater creeks in Algonquin Park but has not been reported from adjacent drainages.

The genus *Lampsilis* exhibits considerable variation in this section. The most "typical" specimens of *L. radiata* have been collected in the Ottawa River, although it has been found in other waters of Ontario.

Summary

1. The pearly freshwater mussel fauna of Ontario comprises 45 species and 18 varieties and of these the greatest number are to be found in the southernmost waters.

2. Seven species are confined in Ontario to Lake Erie, Lake St. Clair, Detroit River, and their drainages.

3. Our present knowledge of the distribution of these mussels, as shown briefly by the list, indicates that the fauna is probably composed of different elements having different affinities and origins. During post-glacial time, when Ontario waters were connected with the Mississippi drainage and with the Atlantic drainage, elements of the Ontario naiad fauna probably migrated from the south and from the east.

Doubtful Species

Anodonta imbecillis Say

This species is recorded from the Michigan side of Lake Erie but as yet we have seen no authentic specimens from Ontario.

Anodonta pepiniana Lea

The status of this species is still in doubt. It may be only a stunted form of *Anodonta grandis*. It has been reported from Lake Superior, from "upper and middle St. Lawrence drainage."

Dysnomia sulcata delicata (Simpson)

This variety is known to occur in the Detroit River but as yet there are no definite records from the Ontario side.

Fusconaia subrotunda (Lea)

The species has been recorded for the Detroit River but so far we have seen no specimens from Ontario. One specimen (NMC 2417) from the Grand River, originally recorded as this species, is *Pleurobema cordatum coccineum*.

Fusconaia undata (Barnes)

This species also has been recorded for the Detroit River but so far we have seen no authentic specimens from Ontario. Two specimens from Port Dover, Lake Erie (NMC 2457) were identified as this species by Simpson, but Walker, who examined them later, decided that one of them was "*Quadrula flava*" and labelled the other "may be *Quadrula undata* Bar. but very doubtful, rather *flava*". Dr. Henry Vander Schalie, to whom this lot was submitted, assures us that both specimens should be referred to *flava parvula*.

Lampsilis ochracea (Say)

All Ontario records appear doubtful.

Leptodea leptodon Raf.

Is recorded for Detroit River. We have seen no authentic Ontario specimens.

Quadrula pustulosa schoolcraftensis (Lea)

Recorded (as *pustulosa prasina*) for Lake Erie by Goodrich and Vander Schalie. We have seen no authentic Ontario specimens.

Simpsoniconcha ambigua (Say)

The species is known to occur in Lake Erie and the Detroit River but as yet there are no definite records from the Ontario side.

FAMILY MARGARITANIDAE:

Margaritana margaritifera (L.)

Recorded for the St. Lawrence River in Quebec but not known for Ontario.

Erroneous Records

The following species have been recorded for Ontario in error:

Actinonaias ellipsiformis (Conrad)

Simpson lists this species for "Southern Canada" and it is recorded by Robertson for the Toronto region, the record being based on specimens in the Brodie collection. This collection is now in the Royal Ontario Museum of Zoology and contains no specimens of this species; neither are there any specimens in the National Museum. Moreover, Dr. Vander Schalie is convinced that this is an error and suspects that specimens of *Micromya iris* were mistaken for this species, which is readily done by one not familiar with the group.

Elliptio crassidens (Lamarck)

Nicholson (1872) lists this species for Toronto Bay, and Robertson (Nat. Hist. Toronto Region, 1913, p. 292) repeats the Nicholson record, but with a question mark. We have never seen specimens of *E. crassidens* from Toronto Bay or from any other part of Ontario. It seems probable, therefore, that the original record was based upon a misidentification.

Pleurobema clava (Lamarck)

The basis for this record is the citation of Call (Trans. Acad. Sci. St. Louis, Vol. 7, p. 9, 1895) who says in part: ". . . . it ranges, as specimens at hand prove, from western New York to Ottawa river, Canada, thence west to Illinois and south to Middle Alabama . . . . " We believe the Ottawa River record to be erroneous, since the species has never been found there to our knowledge although local collectors have been fairly active both before and since the publication of Call's record. So far we have seen no authentic specimens from Ontario.

TABLE I

Distribution of species

Species	Hudson Bay Drainage	Lake Superior	Lake Superior Drainage	Lake Huron	Lake Huron Drainage	Lake St. Clair	Lake St. Clair Drainage	Detroit River	Lake Erie	Lake Erie Drainage	Niagara River and Trib.	Lake Ontario	Lake Ontario Drainage	St. Lawrence and Trib.	Ottawa Riv. and Trib.
Actinonaias carinata (Barnes)						M	*		M	*	*	*			
Alasmidonta calceolus (Lea)				*		*	*	*	*	*	*	*			*
Alasmidonta marginata (Say)				*	*	*	*	*	*	*	*	9		*	*
Alasmidonta undulata (Say)														*	*
Amblema costata (Raf.)					*	*	*	*	*	*	*				A
Amblema plicata (Say)				*		*		M	*		*				A
Anodonta cataracta Say	The species have been recorded for Ontario. The genus is generally distributed in all drainages.														
Anodonta grandis Say															
Anodonta grandis benedictensis Lea															
Anodonta grandis footiana (Lea)															
Anodonta grandis gigantea (Lea)															
Anodonta implicata Say															
Anodonta kennicottii Lea															
Anodonta marginata Say															
Anodontoides ferussacianus (Lea)				*	*		*		*	*		*	15	*	*
Anodontoides ferussacianus buchanensis (Lea)				*		*			*						
Anodontoides ferussacianus subcylindraceus (Lea)			*	*	*	*	*	*	*	*	*	*	*	*	*
Carunculina parva (Barnes)									*	*					A
Cyclonaias tuberculata (Raf.)							*	M	*						A
Dysnomia rangiana (Lea)						*		*	*						A
Dysnomia triquetra (Raf.)				*		*	*	*	*	*					A
Elliptio complanatus (Dillwyn)	*	*	20	*	*	A?	A?		*?	*?	*	*	*	*	*
Elliptio dilatatus Raf.				*	*		*	*		*	*	*	*?	*	*
Elliptio dilatatus sterkii Grier				*		*			*		*		*?		A
Fusconaia flava (Raf.)					5?		*			*	*				A
Fusconaia flava parvula Grier				*		*			*						A
Lampsilis cariosa (Say)													*		*
Lampsilis fasciola (Raf.)				*		*	*	*	*	*	*				A
Lampsilis radiata (Gmelin)	1		*	*	*		*			*?		*		*	*
Lampsilis radiata borealis (A. F. Gray)	*?		*		*?								*?		*
Lampsilis siliquoidea (Barnes)	*	*		*	*	*	*	*	*	*	*	*		*	*
Lampsilis siliquoidea rosacea (DeKay)	*	15	15	*	*	*			*		*	*	15	*	
Lampsilis superiorensis (Marsh)	*	M	*		*										
Lampsilis ventricosa (Barnes)	*	*?		*	*	*	*	*	*	*	*		*?	*	*
Lampsilis ventricosa canadensis (Lea)	M			*		*			*			*?		*?	*?
Lasmigona complanata (Barnes)	*						.		*?						A
Lasmigona complanata katherinae (Lea)		*?		*											A
Lasmigona compressa (Lea)	*		M		*	*	*	*	*	*	*	*?	*	*	*
Lasmigona costata (Raf.)			15		*	*	*	*		*	*		*		*
Lasmigona costata eriganensis Grier						*			*		*				A
Leptodea fragilis (Raf.)						*	*	*	*	*	*	*			*
Leptodea fragilis lacustris F. C. Baker						*			*		*?	*			A
Ligumia nasuta (Say)				*		*		*	*	*	*	*	*		A
Ligumia recta (Lam.)				*	*	*		*	*	*	*	*		*	*
Ligumia recta latissima (Raf.)										*			*		*
Micromya fabalis (Lea)							*	*	*						A

TABLE I—*Concluded*

DISTRIBUTION OF SPECIES—*Concluded*

Species	Hudson Bay Drainage	Lake Superior	Lake Superior Drainage	Lake Huron	Lake Huron Drainage	Lake St. Clair	Lake St. Clair Drainage	Detroit River	Lake Erie	Lake Erie Drainage	Niagara River and Trib.	Lake Ontario	Lake Ontario Drainage	St. Lawrence and Trib.	Ottawa Riv. and Trib.
Micromya iris (Lea)					*	*	*		*	*	*	*	M		
Micromya iris novi-eboraci (Lea)					*	*	*	*	*	*	*			*	
Obliquaria reflexa (Raf.)							*		*	*					A
Obovaria leibii (Lea)						*	20?	*	*	*					A
Obovaria olivaria (Raf.)					*	*	*	*	*	20	*			*	*
Pleurobema cordatum coccineum					M	*	*	*	*	*	*		20?		A
Pleurobema cordatum pauperculum						*			*		19?				A
Proptera alata (Say)	*?			*		*	*	*	*	*	*	*			*
Proptera alata megaptera (Raf.)				*?			*		*?	*	*				*
Ptychobranchus fasciolare (Raf.)						*	*	*	*	*	*				A
Quadrula pustulosa (Lea)							*		*	*	*				A
Quadrula quadrula (Raf.)							*		*	*	*				A
Strophitus rugosus (Swain.)				*	*	*?	*	M	*	*	*	*?	M	*	*
Truncilla donaciformis (Lea)						*			*	*	*?				A
Truncilla truncata (Raf.)						*	*		*	*					A
TOTALS															
Species	8	4	5	16	16	23	27	23	30	29	24	13	8	12	17
Species (? Record doubtful)	1	1	0	0	1	1	1	0	2	2	1	2	3	0	0
Varieties	2	1	3	7	4	11	4	2	10	4	6	3	3	3	4
Varieties (? Record doubtful)	1	1	0	1	1	0	0	0	1	0	2	1	2	1	1

**Specimens in National Museum of Canada or Royal Ontario Museum of Zoology.*
M—*In University Museums, University of Michigan.*
1, etc.—*Record in literature listed.*
?—*Doubtful record.*
A—*Absent.*

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SOME OBSERVATIONS ON THE OVERWINTERING OF CERTAIN HELMINTH PARASITES OF SHEEP IN CANADA[1]

By Henry J. Griffiths[2]

Abstract

The helminths, *Meniezia expansa*, *Ostertagia circumcincta*, *Nematodirus filicollis* and *Trichostrongylus colubriformis* demonstrated the ability to survive a normal Canadian winter and to cause infection of sheep when ingested during the following spring. Temperatures during the four-month period ranged from −15° F. to 52.5° F. It is concluded that pastures cannot be considered free from these helminths after exposure to such temperatures.

Introduction

While it is an accepted principle of control of helminth parasites in domestic animals that prevention of infection is greatly preferable to destruction of the helminth after it has gained access to the host, it is true that most reliance has so far been placed on anthelmintic medication. This is unfortunate for many reasons, among the most important of which are that few anthelmintics are efficient and all are more or less toxic, and very often much damage has been done by the young parasite before it becomes susceptible to the action of the drug.

This state of affairs has arisen largely because of our lack of knowledge of the bionomics of the helminths and our consequent inability to apply rational, scientific methods of control. At the present time, however, with the limited knowledge available, much can be done by general sanitation, light stocking, pasture rotation, bare lots, and so on, in reducing infestations.

The importance of a helminthic infestation almost always depends upon the number of worms present, and, as no worm can multiply entirely inside the body, the extent of the infection depends ultimately on the number of eggs or larvae on the pasture. It is accordingly essential to have an adequate knowledge of the conditions under which these eggs and larvae will survive in the free state. The following observations on the effects of a Canadian winter on some of these parasites, are presented as helping to throw some light on the free life of these animals.

Results were obtained incidentally, while attempting some pure-strain infestations of sheep with a single helminth. Records of the previous season's experiments on these plots, and the biological data revealed, seemed of sufficient significance to be recorded.

Experimental

Plots

The plots utilized in these trials were small, averaging about 350 sq. yd. each. They were well fenced, and in the early part of the summer yielded fair pasture, comprising a mixture of the following common grasses: white

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Dutch clover, red top, timothy, Kentucky bluegrass, couch grass and cocksfoot. After a short period of pasturing, supplemental feeds were supplied owing to the exceedingly heavy concentration of sheep on so small an area. Experimental plots were separated from each other by a plot which was not in use. This precaution avoided danger of fecal contamination.

Stock

The lambs employed in this work were from the experimental flock of this Institute. They were born and raised under as helminth-free conditions as practicable, being permitted to suckle their mothers only at certain periods of the day, following the washing of the ewe's udder. Under these conditions lambs have been raised free of bursate nematodes although, in most cases, coccidiosis and an infestation of *Strongyloides papillosus* were not eliminated.

Lambs raised under these conditions have not shown the normal growth and weight that would be observed with lambs running with their mothers at pasture during the summer. However, the lambs have been in good health and have not shown any deficiency symptoms or other physiological disorders.

Fecal Tests and Post-mortem Examinations

Feces were taken, when necessary, directly from the rectum of the individual. The usual centrifugal methods were employed for diagnostic purposes. Post-mortem examinations were carried out in the usual routine by sedimentation of the contents of the intestine; the helminths obtained were preserved in 70% alcohol.

Observations and Results

Plot I. During the summer of 1935, this pen pastured a ewe and a yearling which were on salt lick trials. Throughout the summer fecal tests revealed the sheep to be harboring at least two species of strongyles, and on one occasion tapeworm ova were recorded. These sheep were removed from pasture early in October, 1935. On May 27, 1936, two lambs were put out to pasture in this pen. One lamb was fed a culture of *Haemonchus contortus* larvae, the other being used as a control. Previous to use on this work the lambs were tested and found positive for coccidia and *Strongyloides papillosus* only. On June 6, fecal examination showed the presence of *S. papillosus* and coccidia. On June 30, the experimentally infected lamb showed ova of two species of strongyles and coccidia, the control lamb yielding ova of two species of strongyles, of *S. papillosus* and of a capillarid, coccidia oöcysts also being present. (At this date, June 30, the feces of five control lambs were examined and found to yield only ova of *S. papillosus* and oöcysts of coccidia.) On July 22, fecal tests were made of the two experimental lambs, and analysis revealed the ova of *Nematodirus* sp., strongyles (probably *H. contortus*), and coccidia oöcysts. This test was repeated on July 23 with identical recordings. This infestation increased and post-mortem results from these lambs were as follows:—

Lamb experimentally infected with *H. contortus:* Killed August 28, 1936	Control lamb, killed July 30, 1936
Ostertagia circumcincta	*O. circumcincta*
Nematodirus filicollis	*N. filicollis*
Haemonchus contortus	*Trichostrongylus colubriformis*
Moniezia expansa	*M. expansa*

Plot II. Throughout the summer of 1935 this plot pastured a ewe, a yearling and a lamb on salt lick experiments similar to those in Plot I. Numerous fecal examinations throughout the summer showed these individuals to be harboring a heavy infestation of helminths, tapeworm ova and four different species of nematode ova being observed. On September 30, 1935, the lamb died, post-mortem examination yielding *Trichostrongylus* sp., *Monodontus trigonocephalus*, and *Chabertia ovina.* During the early part of October, 1935, the ewe and yearling were removed from pasture to winter quarters.

On May 27, 1936, two lambs were pastured in this pen, one animal being fed a pure culture of infective larvae of *C. ovina*, the other being kept as a control. The lamb drenched with the infective larvae died during the last week in June. Unfortunately it was impossible to make an autopsy. Fecal examination of these two lambs on June 11, 1936, revealed the presence of coccidia oöcysts only. On June 30, the control lamb yielded ova of one species of strongyle and of *S. papillosus* on fecal test. (Five control lambs were tested and found to yield only *S. papillosus* ova and coccidia.)

On July 22, 1936, the remaining lamb, on fecal examination, showed heavy infestation of *Moniezia* sp., and ova of a species of strongyle. On July 28, 1936, this lamb was slaughtered, an autopsy yielding the following helminths: *Ostertagia circumcincta*, *Nematodirus filicollis*, *Trichostrongylus colubriformis*, *Moniezia expansa*, *Strongyloides papillosus.*

Plot III. This plot also was utilized for salt lick trials during the summer of 1935, pasturing a ewe, a yearling and a lamb. Numerous fecal examinations of these individuals throughout the summer showed them to be harboring three species of strongyles and tapeworms. These sheep were removed from pasture early in October, 1935.

Two lambs were pastured in this pen on May 27, 1936. Fecal tests on these animals on June 11, 1936, showed an infestation of coccidia only. On June 30, 1936, the examination revealed ova of a species of strongyle and of *S. papillosus* and coccidia oöcysts. (Five controls examined on the same date were found to be carrying *Strongyloides* ova and coccidia only.) Fecal examination of these lambs on July 22, 1936, showed them to be harboring two species of strongyles, *S. papillosus* and coccidia. Post-mortem examination of these lambs yielded the following helminths:

Killed July 22, 1936. *O. circumcincta, N. filicollis, S. papillosus.*
Killed July 24, 1936. *O. circumcincta, N. filicollis, M. expansa.*

Controls

Of the 11 lambs available for experimental purposes, five were kept as controls throughout the summer of 1936, to be used later for other purposes. These five lambs and those selected for use on the experimental plots were raised under identical conditions. They had every opportunity of picking up an infestation of helminths should the technique employed in raising helminth-free lambs have proved unsatisfactory. All the lambs were recorded as carrying *S. papillosus* and a coccidial infection at some time or other and, consequently, these two parasites were disregarded throughout the investigation.

Until June 29, 1936, these five control lambs were kept indoors in concrete pens. On that date the lambs were put out in small paddocks which were known not to have been pastured by sheep for many years. Fecal tests were made of these control lambs at irregular intervals, and at no time were any ova observed except *S. papillosus.* Coccidia oöcysts were observed on most occasions.

Resistance to Winter Temperatures

Certain helminths mentioned in this paper have been shown to possess the ability to resist the low temperatures of a Canadian winter. These helminths not only have the power to withstand comparatively low temperatures, but also appear to possess the ability to withstand freezing and thawing to a considerable extent.

Whether this resistance mechanism is functional in the ovum or the infective larvae is still debatable, but from the observations of other investigators it is probable that infective larvae would withstand the rigors of winter to a far greater extent than would the ova.

The maximum and minimum temperatures for a four-month period of the winter of 1935–36, are charted in Fig. 1. These temperatures were obtained through the courtesy of the Department of Horticulture, Macdonald College, McGill University.

It will readily be noted from the graphs that some sharp rises and falls in temperature were recorded during the four-month period. The maximum and minimum temperatures, as indicated in the graphs, for December, 1935, and January and February, 1936, will be observed to be mostly below 32° F. At several periods during these months the mercury dropped to zero or well below, the minimum temperature recording well below zero for several days at a time in some instances. In the first few days of March the temperature dropped to −11° F. This was followed by milder weather, though at no period during the month did the minimum recorded temperature rise much above freezing point.

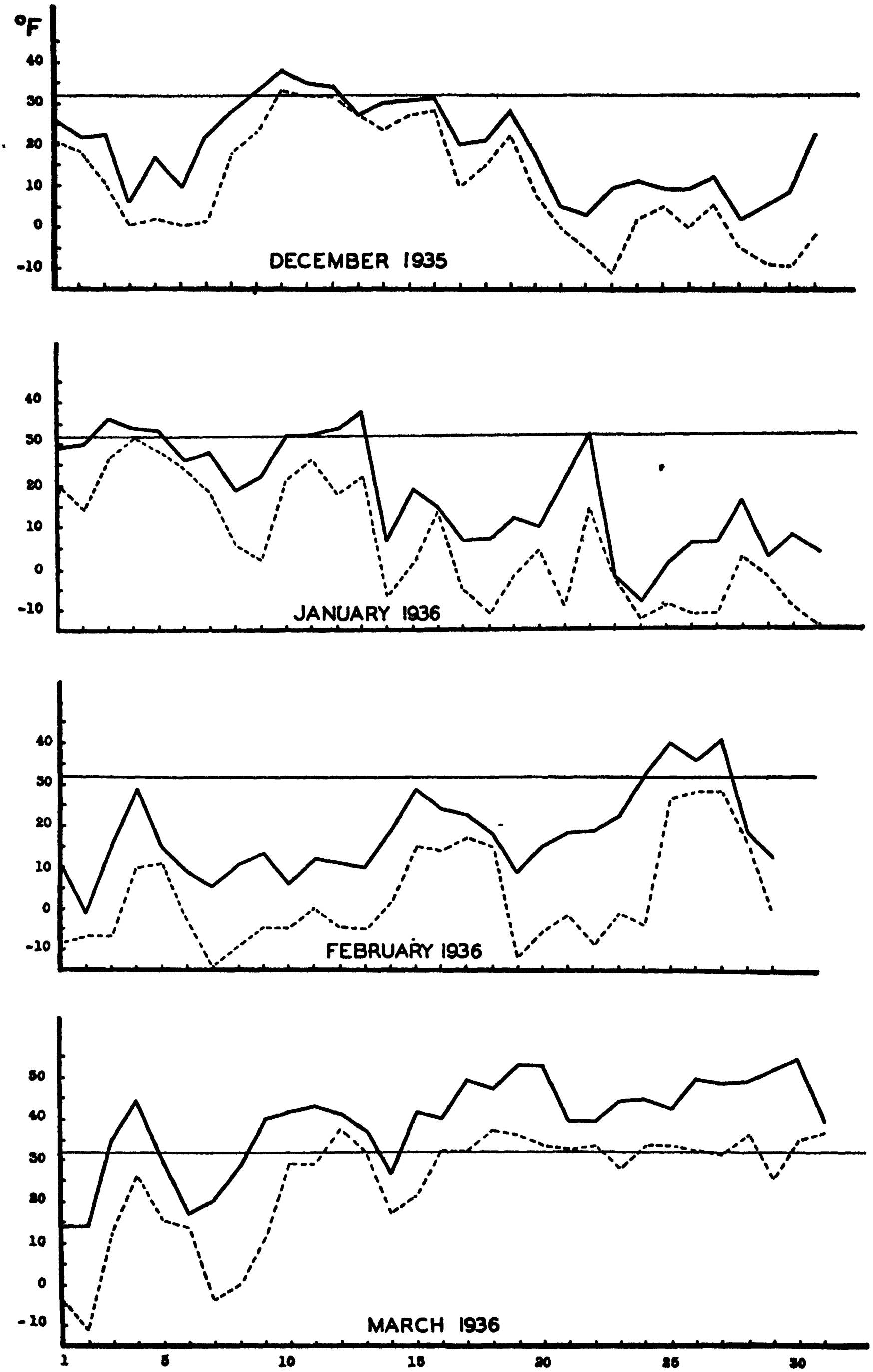

FIG. 1. *Graphs showing maximum and minimum temperatures for a four-month period in the winter of 1935–36. Maximum temperatures indicated by continuous line, minimum by dotted line.*

December, January and February were slightly colder than the average for the island of Montreal, although March was warmer (Table I). The precipitation for January and February was normal—both rain and snow—but in December both rain and snow were considerably less than usual, and in March rain was above average while snowfall was below average. It seems therefore that the winter of 1935 in this district was slightly more severe than normal and that the worms which survived this winter might be expected to survive any normal winter in the Montreal area.

TABLE I

TEMPERATURE AND PRECIPITATION

—	December		January		February		March	
	Average	1935	Average	1936	Average	1936	Average	1936
Temperature, ° F.								
Maximum	26	19.3	21.0	17.9	22.0	17.9	33.0	39.0
Mean	20	14.8	13.0	11.9	14.0	10.3	26.0	31.55
Minimum	13	10.4	5.0	6.0	7.0	2.72	18.0	24.11
Total precipitation	3.74	1.245	3.75	3.27	3.17	3.05	3.52	4.41
Snowfall, in.	23.7	9.75	28.5	28.5	24.4	25.5	24.4	11.25

Average figures taken from Canada Year Book for 1931. (Total precipitation includes snowfall calculated at 10 in. snow = 1 in. rain.)

Discussion

Up to the present, few data have been available on the lethal effect of Canadian winter temperatures on the non-parasitic stages of the common helminth parasites of sheep while on pasture land. It has been possible, from the information available on the bionomics of certain parasites, to predict the improbability of their survival throughout the winter. Undoubtedly the survival of helminths would not depend merely on one factor such as temperature, but on a complex of factors, such as precipitation, period of exposure to a certain temperature, snow-cover, type of herbage, location of pasture and many other factors.

The small number of experimental animals employed may be criticized but the observations recorded were significant, not relative to the numbers employed, but to the fact that the animals used must have been raised without possible chance of bursate nematode infestation. *Strongyloides papillosus* was observed in most cases, and on account of the certainty with which the ovum can be identified, was disregarded throughout the experiment. It has been found practically impossible to raise lambs free of this parasite.

Chance contamination of the lambs exposed to the "pure-line" infection in each pen could possibly have occurred during the culturing and preparation of the infective larvae for drenching. This chance, however, was eliminated by the use of a control lamb in the two pens where pure line infections were attempted.

Conclusion

The following common helminths of sheep demonstrated the ability of being able to survive a normal Canadian winter and to cause an infestation within the sheep when ingested at pasture during the following spring: *Moniezia expansa*, *Ostertagia circumcincta*, *Nematodirus filicollis*, *Trichostrongylus colubriformis*.

These species were able to withstand temperatures ranging from −15° F. to 52.5 ° F. encountered during the four month period.

It is apparent that pastures cannot be considered as free from these helminths after exposure to climatic factors experienced during one normal Canadian winter.

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THE TAXONOMY OF *DIPHYLLOBOTHRIUM LATUM* (LINNÉ, 1758) IN WESTERN CANADA[1]

BY ROBERT ARNOLD WARDLE[2] AND ELLEN LOUISE MCCOLL[3]

Abstract

On the basis of accepted criteria for the differentiation of species of *Diphyllobothrium* Cobbold, diphyllobothriid material from humans and dogs in Manitoba, one of the three endemic areas of diphyllobothriosis in North America, is adjudged to be homogeneous and to be specifically identical with authenticated *D. latum* material from Russia, Finland and Japan. The classificatory value of various characteristics of *D. latum* and the extent of variability of these characteristics in Canadian material are discussed.

Introduction

In spite of a long and intimate sociological association, the physiological differences between man and dog are sufficiently profound to discourage the common possession of identical species of parasites. *Diphyllobothrium latum* (Linné, 1758) is, with the possible and doubtful exception of *Dipylidium caninum*, the only tapeworm found commonly and habitually in both dog and man, and if we except a few forms of restricted distribution that stray to man occasionally from the dog, it is the only parasite, external or internal, common to both dog and man and apparently equally at home in either host.

It is exceptional also among tapeworms in its wide range of definitive hosts, a range that includes at least seven species of *Felis*, four species of *Canis*, four species of seal, three of bear, one of whale, mongoose, fox, mink, domestic pig and man.

Such catholicity of host adoption, when shown by a member of a group of parasites notoriously restricted in their choice of definitive hosts, should raise the suspicion that the characters chosen arbitrarily for the delineation of this species are inadequate, and that *latum* is in fact not a species at all but a group of closely related physiological species in course of evolutionary divergence. The point has not been raised previously, to our knowledge, and no close comparison appears to have been made between alleged *latum* specimens from different host species within a restricted area.

Relatively common in sled dogs in certain districts of Manitoba is a form of tapeworm sufficiently resembling the accepted descriptions of *latum* to justify identification with it (4, 5, 9, 12). It is not uncommon also among ranch-raised foxes and mink. To what extent it occurs among wolves, bears and wild foxes is undetermined; nor is its distribution among the human population accurately known. Fecal examinations of hospital patients, university students, reservation Indians, and so forth, suggest an extremely low incidence of infestation, probably not exceeding 2%. Apart from people of Indian blood, the population is predominantly French, Scottish, Ukrainian, Icelandic and Jewish in ancestral origin, and the consumption of raw fish is not a common custom. A few cases of human diphyllobothriosis are noted each year, however, by medical practitioners, chiefly among Jewish housewives, and there is reason to attribute some of the cases recorded in other cities of North America to initial infection from fishes of Manitoba origin.

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Taxonomic Criteria

The validity and limitations of the characters used in distinguishing species of tapeworms have been discussed previously (10). For the present comparison the characters selected as being least likely to be influenced by variations of the host environment were the uterine shape, the position of the common genital pore, and the "height", *i.e.*, the dorso-ventral measurements in a 16μ-thick transverse section of a gravid proglottis, of the cuticle, subcuticle, cortex, vitellaria, muscle zone, medulla, testes and cirrus sac, as compared with the height of the proglottis itself. Observations were also made upon the shape and size of the scolex, strobila and proglottids.

The material, comprising specimens from humans in Manitoba, northern Russia, Finland and Japan, and from dogs in Manitoba and northern Russia, had been fixed in formalin, and for purposes of microscopical examination was stained by Delafield's haematoxylin technique, dehydrated in dioxan, cleared in methyl salicylate and mounted in xylol-balsam.

Scolex and Strobila

The scolex of *Diphyllobothrium latum* is described by European authors as spoon-shaped (spatulate), almond-shaped (amygdalate) or olive-shaped (olivate), and approximately 2 mm. long by 1 mm. in maximum breadth.

The shape and size of the scolex and strobila of a living tapeworm just removed from the host and observed in an artificial medium are continually altering owing to the absence of skeletal tissue. The extent of the change is determined by the chemical nature, hydrogen ion concentration and temperature of the medium, and by the length of time that the tapeworm has been divorced from its host. This question has already been discussed (9, 11), but the point may be stressed that even if the fixative employed were to kill the tapeworm instantaneously, which in actual practice never happens since the action of the fixative is impeded by the cuticle, no two specimens will be identical in shape and size after being killed, even if approximately so when alive. Precise descriptions of the shape and size of the scolex and strobila are therefore unreliable. On the other hand, the post-mortem shape and dimensions are determined by the extent of muscular relaxation at death, so that, among a range of specimens killed in the same way by exposure to a weak fixative such as 5–10% formalin, it should be possible to determine the limiting boundaries of shape and size, since the extent of muscular relaxation will vary only within certain limits.

Among the specimens obtained from dogs and foxes in Manitoba,—approximately 100 specimens—the shape of the scolex varied from narrowly lanceolate to broadly spatulate (Fig. 1). A similar range of shape was seen among collections of 20–27 scolices from individual dogs. The commonest type among local diphyllobothriid material is the clavate (Fig. 2), with its maximum width at one-third of the length from the bluntly rounded apex of the scolex. There is a tendency, probably correlated with a progressive deterioration of the scolex musculature, for the shape of the scolex to change, as the worm ages, from an active lanceolate or digitate type to a relatively inactive clavate or spatulate type.

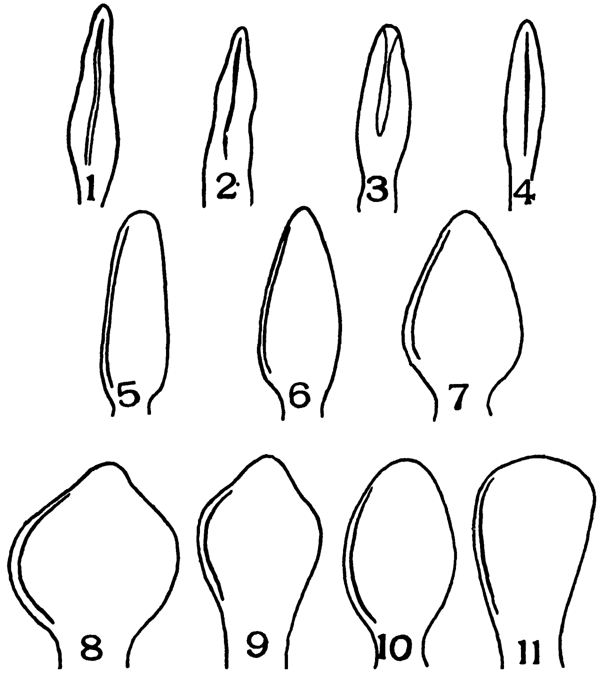

FIG. 1. *Range of scolex shape of Diphyllobothrium latum in Manitoba material. 1, 2, 3, 4 scolices less than 25 hours old; 5, digitate; 6, lanceolate; 7, ovate; 8, cordate; 9, clavate; 10, olivate; 11, spatulate.*

Among 29 scolices taken from a dog infested for 75 days, the lengths varied, after formalin fixation, from 1.326–2.400 mm. with a mean value of 1.700 mm.; the breadths varied from 0.625–1.000 mm. (maximum breadth accompanying minimum length) with a mean value of 0.747 mm. Among 24 scolices from a dog infested for 56 days the mean length by breadth values were 1.523 × 0.926 mm. In one scolex from a Russian dog, infested 36 days, the shape was clavate, the dimensions 1.25 × 0.75 mm. Unfortunately only four scolices from man were available. Two Russian scolices were spatulate, with mean dimensions of 1.5 × 0.75 mm. Two scolices from man in Manitoba were clavate with mean dimensions 1.25 × 0.9 mm. Magath (3) describes one scolex from man in Minnesota as 1.4 × 0.5 mm. in dimensions, and figures it as midway between digitate and olivate.

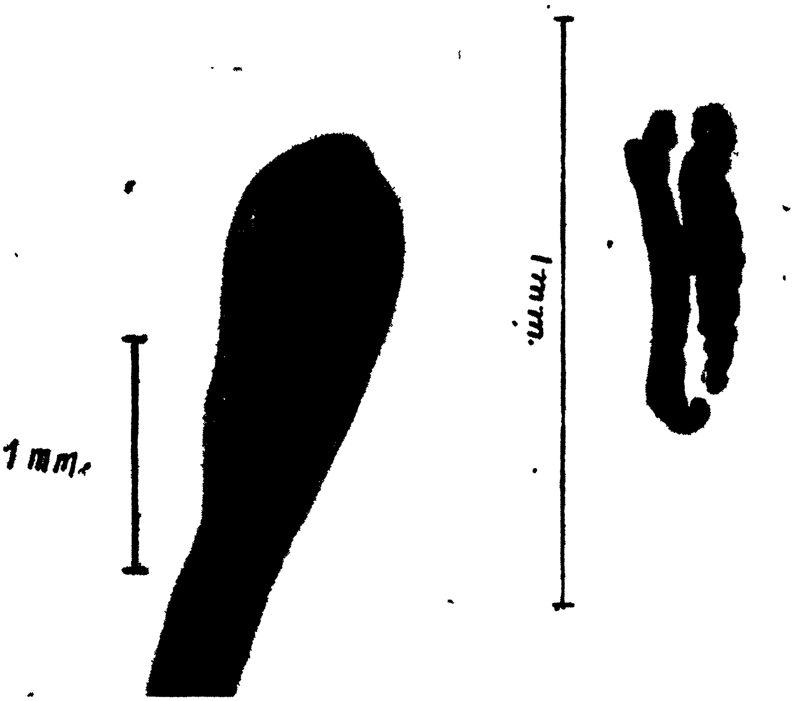

FIG. 2. *Scolex of Diphyllobothrium latum from cat, Winnipeg, seen in surficial view and in transverse section.*

The shape of 27 scolices removed from the host (kitten) within 25 hr. after initial infection was invariably acutely lanceolate (Fig. 1) and the mean dimensions 1.66 × 0.5 mm. Presumably, therefore, no growth of the scolex takes place after the plerocercoid larva reaches the definitive host. This appears to be the case in tapeworms generally. The scolex is necessary to anchor the small and muscularly weak early strobila, but in the older strobila it serves little purpose, the tapeworm maintaining its station by muscular tonus.

The neck region extends from the posterior margins of the bothria to the first interproglottid boundary. Although the most important physiological region of the tapeworm, it is histologically the most obscure, since it does not react with the usual microtechnical reagents. Its posterior margin—the first interproglottid groove—is difficult to fix with precision, so that the extent to which the neck region corresponds with the original plerocercoid body is uncertain. Among the 27 individuals up to 25 hr. old, mentioned above, the lengths—including the scolex—varied from 6–24 mm., with a mean of 10.0 mm. Segmentation begins usually at about 25 hours after initial infection. Among five 30-hr. strobilae, whose mean length was 45 mm., the neck region varied from 3.5–4.0 mm. in length and can represent therefore only the anterior third of the plerocercoid. Among 23 specimens from a dog infested 56 days, the neck lengths varied from 1.4–2 6 mm. with a mean value of 2.2 mm. Magath (3), however, estimated the neck length of a single specimen from man as 8 mm.

Segmentation of the early strobila between 25 and 72 hr. old appears almost simultaneously along the whole strobilar length, the primary strobila being thus formed as in other families of pseudophyllidean tapeworms—Ptychobothriidae, Amphicotylidae—where no neck is present. The absence of a neck from certain diphyllobothriid species parasitic in pinnipede Carnivora and ascribed to the genus *Diphyllobothrium* Cobbold would suggest that this method of primary strobila formation was the original method in *Diphyllobothrium* also and that the neck is, from an evolutionary point of view, a new structure. It would be a matter of great interest to know whether, in such apparently primitive diphyllobothriids, the secondary strobila is formed as in Pseudophyllidea generally, by division of pre-existing proglottids or, as in *Diphyllobothrium latum*, by origin of new proglottids from the neck region.

The early strobila is completely segmented by the 72nd hour after initial infection. It consists of "long" proglottids, *i.e.*, proglottids with a length of 1.5–2.0 times the breadth. The proglottids are markedly craspedote and the terminal proglottis has a characteristic bluntly rounded posterior margin. In superinfested dogs such primary strobilae may be found several months after initial infection. The alleged species *parvum* of Stephens (7) appears almost certainly to have been a primary strobila of *latum* about nine metres in length, occurring in man, but under the conditions of light infestation such as occur usually in human hosts it is probable that the primary strobila passes fairly rapidly into the secondary phase.

The new proglottids of the secondary strobila are at first linear, much wider than long; as they mature they become quadrate; under conditions of ample nutrition they remain linear, the breadth being three to four times the length. Under conditions of superinfestation in dogs the characteristic shape of the secondary proglottids is quadrate, or nearly so, but strips of long and craspedote proglottids may interrupt the sequence of quadrate proglottids. Such recurrence of primary strobilar characters may arise from a slowing down of genital development and a release of material for tissue growth. Under conditions of light infestation and ample nutrition the proglottids are linear, because the nutritional demands of the reproductive system limit the surplus of material available for tissue growth. It is probable that a survey of tapeworms in general would show that when the number of individuals per host is habitually low, in consequence of premunition, and the sexual development rapid, the proglottid shape is usually linear.

The most extensive data concerning the size of the strobila in *D. latum* in Europe are those of Tarassow (8) who found that, among 405 specimens from humans in the Karelia-Murmansk-Leningrad districts of U.S.S.R., the strobilar length varied from one to seventeen metres, the majority of the specimens being 8-10 metres in length. Tapeworm material obtained from human hosts by vermifugation is usually incomplete, and accurate measurements of strobilae from North American human hosts are not available. Scattered observations suggest that they rarely exceed two metres in length. In dogs they are smaller still. In superinfested dogs in our experiments the mean strobilar length varied from 25.6 cm. (32 days after initial infection) to 169 cm. (98 days after initial infection).

On the whole, therefore, variations in shape and size of the scolex and strobila of *Diphyllobothrium latum*, although of considerable physiological interest, have little taxonomic value.

Internal Dimensions

There has been a tendency in recent years for students of tapeworm taxonomy to provide meticulous micrometric measurements of such internal structures as the cirrus sac, testes, germarium, vitellaria and genital ducts, and exhaustively detailed descriptions of the cuticle, subcuticle, musculature, parenchyma and so forth. Valuable as such ponderous and scholarly descriptions must be to the student of comparative histology, it may be doubted whether they are of like value to the taxonomist, although certainly preferable to the inadequate descriptions that have accompanied the announcement of many alleged new species. A tapeworm has no system of skeletal tissues such as serve in other animals to keep the dimensions of anatomical structures between definable limits, and such structures as the cirrus sac, uterus, and genital ducts have markedly contractile muscular walls that make them extremely susceptible to the distorting effects of technical reagents. A tapeworm killed quickly by suspension in a hot aqueous medium such as 10% formalin or corrosive sublimate-acetic acid, or even hot water alone, will increase in length by 50–60% owing to the relaxation of the longitudinal musculature. Absolute dimensional measurements of tapeworm structures are therefore of doubtful value unless the material under comparison has received identical treatment. On the other hand, relative measurements where the length or breadth or height of the proglottis is used as a standard of comparison have considerable value when applied to a range of material killed and preserved in like fashion.

The data in Table I are mean values of the height, *i.e.*, the vertical diametrical measurements, of the cuticle, subcuticle, cortex, vitellaria, muscle zone, medulla, testes and cirrus sac, expressed as percentages of the height of the proglottis measured at a point midway between the middle line and the lateral margin, except in the case of the cirrus sac whose height is compared with the height of the proglottis in the middle line.

TABLE I

DORSO-VENTRAL DIMENSIONS OF PROGLOTTIS STRUCTURES EXPRESSED AS PERCENTAGES OF THE PROGLOTTIS THICKNESS. LENGTH, BREADTH AND THICKNESS OF THE PROGLOTTIS ARE GIVEN IN MILLIMETRES

Origin	Lgth.	Bdth.	Th.	Cut.	Sc.	Cort.	Vit.	Musc.	Med.	Cs.	Test.
Man, Russia	4.18	10.0	0.700	0.7	4.0	24.0	14.0	7.0	30.0	0.66	21.0
Man, Finland	2.90	8.0	.760	0.4	8.0	14.0	11.0	16.0	21.0	.47	15.0
Man, Japan	3.22	10.0	.661	1.06	6.8	15.0	10.6	16.0	22.7	.43	16.4
Mean values				0.72	6.2	18.0	12.0	13.0	24.6	.52	17.5
Man, Winnipeg,	2.47	7.5	.856	0.94	6.0	23.5	9.4	11.7	17.6	.50	14.0
Man, Winnipeg	3.54	10.0	.590	0.34	8.5	11.0	13.6	22.0	33.2	.51	24.6
Man. Winnipeg	1.97	7.0	174	0.28	7.0	16.7	9.8	15.4	21.0	.57	18.1
Mean values				0.52	7.2	17.1	11.0	16.3	24.0	.53	19.0
Dog, Russia	5.15	5.0	.850	1.17	4.7	27.5	11.7	11.7	17.6	.75	17.6
Dog, Churchill	1.05	3.0	.978	1.40	12.0	14.3	12.2	14.3	21.1	.31	13.8
Dog, Winnipeg	7.87	6.5	.540	2.80	11.1	14.8	11.1	3.7	22.2	.60	14.0
Mean values				1.79	9.3	19.5	11.0	9.9	20.3	.55	15.1

Cut. cuticle, Sc. subcuticle, Cort. cortex, Vit. vitellaria, Musc. muscle zone, Med. medulla, Cs. cirrus sac, Test. testes.

The material compared comprises three specimens from man in Manitoba, three from man in Leningrad, Finland and Japan, and three specimens from dogs in Manitoba (Winnipeg, Churchill) and Leningrad. The material in each case was fixed in formalin, embedded in paraffin, cut to a thickness of 16μ and stained by the iron-haematoxylin technique.

Comparison of data from human material suggests that, despite considerable individual variation in the percentage proportions of the internal structures measured, there is a fundamental similarity, brought out particularly by comparing the mean values of the three foreign specimens with those of the Manitoba specimens. The chief differences are in thickness of cuticle, which might be expected to vary with host gut conditions, and thickness of the muscle zone, which might be expected to vary with the degree of relaxation induced by fixation. Among the specimens from dogs there was greater variation, especially as regards the cuticle, subcuticle and muscle zones, but again the mean values of the three do not depart so widely from those of human material as to suggest any specific differentiation. The material from Churchill, the most northerly location for *D. latum* yet recorded in Canada, consisted of twelve strobilae, believed to be primary, with a mean length of 30 cm. and width of 3 mm. This material showed certain peculiarities, notably the markedly digitate scolices, 1.7 × 0.7 mm. in dimensions, the great proglottid thickness (978μ), extremely thick subcuticle (90μ), thick muscle zone (140μ), small cirrus sac, small testes—scarcely larger than the vitellaria—, with long axes vertical instead of horizontal, and the absence of circum-cloacal papillae which are usually very prominent in material from dogs. In the absence of human material from this area, however, it would be premature to draw any conclusions as to specific or sub-specific distinction concerning this material.

Genital Pore

The descriptions of the genitalia of *D. latum* provided by early authors have been incorporated and added to in the accounts given by Sommer and Landois (6) and by Magath (3) and need not be repeated here.

The term "porus genitalis" is not clearly defined by earlier authors and actually in *latum* there are three such pores, the vagina and cirrus sac opening separately into a genital cloaca which itself has an exit to the exterior. The

TABLE II

LOOP COUNTS, EACH SIDE OF THE MIDDLE LINE, FOR THE ANTERIOR UTERUS (Ant. ut.) AND POSTERIOR UTERUS (Post. ut.) AND RATIOS OF DISTANCE (Gp.) OF COMMON GENITAL PORE FROM ANTERIOR PROGLOTTID MARGIN TO PROGLOTTID LENGTH (Lgth.). THE VALUES ARE MEAN FOR *n* CONSECUTIVE PROGLOTTIDS

Origin	*n*.	Ant. ut.	Post ut.	Lgth., mm.	Gp., mm.	Ratio
Man,						
Russia	20	5.2 : 4.7	2.05 : 2.55	5.67	1.35	0.234
Russia	20	5.9 : 5.5	2.80 : 3.20	4.18	0.857	.205
Finland	20	6.3 : 6.0	1.80 : 2.30	2.90	0.417	.144
Japan	20	4.7 : 4.3	1.40 : 2.00	3.22	0.825	.256
Manitoba	20	4.2 : 4.5	1.30 : 1.50	2.47	0.399	.162
Manitoba	10	4.1 : 4.4	2.40 : 3.40	3.54	0.810	.229
Manitoba	20	4.2 : 4.5	2.70 : 2.90	1.97	0.386	.196
Manitoba	20	3.8 : 3.4	1.30 : 2.00	1.70	0.308	.181
Manitoba	20	3.9 : 3.4	1.10 : 1.70	1.38	0.252	.182
Manitoba	20	4.9 : 5.1	1.90 : 2.20	2.07	0.330	.159
Manitoba	20	4.9 : 5.2	2.00 : 2.50	3.03	0.635	.209
Manitoba	20	5.0 : 4.7	— —	1.70	0.312	.184
Dog,						
Russia	20	7.2 : 7.3	2.9 : 2.7	5.15	1.210	.235
Gimli	12	5.6 : 6.3	1.5 : 1.3	2.30	0.308	.134
Winnipeg	7	8.0 : 7.9	3.1 : 3.9	7.87	2.012	.255
Winnipeg	12	6.2 : 6.0	2.8 : 2.4	1.60	0.324	.202
Churchill	9	4.0 : 4.0	— —	1.05	0.184	.175
Fox						
Gimli	20	5.3 : 5.6	2.7 : 2.6	3.06	0.572	.187

term will be used here to imply the central point of the common genital pore or cloacal aperture. This is frequently placed vertically ventral to the male pore but occasionally, due to distortion of the cuticle, it is shifted to the female pore region. Its position is usually described in terms of its distance from the anterior margin of the proglottis and is a favorite taxonomic character with students of Diphyllobothriidae. In European specimens it is usually said to be situated at about the anterior quarter of the proglottid length. In the case of a Minnesota specimen Magath (3) gives a distance of 0.44 mm. from the anterior margin of a segment 2.5 mm. in length, that is to say a ratio to proglottid length of 0.18.

Table II gives this ratio of genital pore position for a series of consecutive proglottids, usually 20, in material from man in Russia, Finland, Japan, Manitoba (eight specimens) and from dogs in Russia and Manitoba (Winnipeg, Gimli, Churchill). In the material as a whole the ratio varied from 0.134 (Gimli dog) to 0.255 (Winnipeg dog) with a general mean of 0.196. In Manitoba human material the ratio varied from 0.162–0.229 with a mean of 0.188; in Manitoba dog material it varied from 0.134–0.255 with a mean of 0.19. The material from a fox had a ratio of 0.187; from a dog at Churchill, a mean ratio of 0.175. On the whole therefore the common genital pore in Manitoba material is nearer the anterior margin of the proglottis than it is in Russian or Japanese material, but the difference of position is too slight to constitute a valid specific differentiation.

Uterine Shape

The uterus of *Diphyllobothrium latum* is a tube fixed between its entrance from the oötype and its exit to the exterior, and thrown into a series of coils or "loops". The number of these loops on each side of the middle line has long been a taxonomic character for species of *Diphyllobothrium*. The steps in the development of this looped condition have not to our knowledge been described and may be briefly discussed here.

In a specimen from man in Leningrad, the anlage of the uterus first appears at 50 mm. from the scolex tip, that is to say between the 250th and 300th proglottids. In the next 156 proglottids, a distance of 165 mm., the uterus appears as a straight, faintly staining structure, presumably tubular, with the anterior end swollen and bent ventrad upon itself (Fig. 3, A) although the apparent swollen extremity may be the anlage of the cirrus sac. By elongation through growth, the straight uterus becomes a loose corkscrew which persists through the next 84 proglottids (Fig. 3, B). Continued growth brings about a lengthening of the corkscrew coils, producing a zigzag pattern that persists through the next 80 proglottids (Fig. 3, C). As eggs begin to pass from the oötype into the uterine tube the somewhat angular bends become rounded, closer together, loop-like and approximately parallel, but directed slightly dorsad and ventrad from the horizontal plane. A distinction now appears between an anterior set of loops—large, approximately parallel, non-stainable, containing eggs, each with a single, longitudinal wrinkle—and a posterior set of loops—small, irregularly arranged, stainable with haematoxylin, containing eggs whose shell, in mounted specimens, is usually intact or occasionally in a condition of general collapse. The wrinkling of the eggs is due to dehydration by the reagents used in microtechnical preparation. In weak formalin or in water no such wrinkling is shown by extruded eggs; in alcohol of 70% or greater strength, a single longitudinal wrinkle appears immediately. Eggs in the posterior uterus appear to have no operculum, whereas those in the anterior uterus are operculated; the distortion of the egg shell observed among the latter eggs seems in some way to be correlated with the presence of the operculum.

The loops of the posterior uterus are difficult to count, often distinguishable only with difficulty, and although used by Faust *et al.* (1), are commonly ignored by systematists.

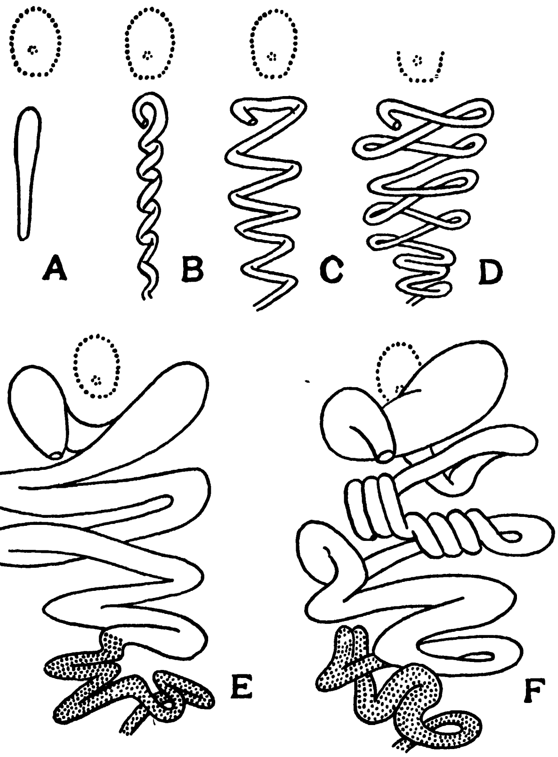

FIG. 3. *Stages in the development of the uterus of Diphyllobothrium latum. The shaded area indicates the posterior uterine region.*

The primary uterine pattern is thus a series of parallel and lateral loops, whose anterior members have thin, chitinoid and non-stainable walls enclosing eggs wrinkled longitudinally (in mounted specimens), and whose posterior members have thicker, epithelioid, stainable walls enclosing eggs that are either not distorted or else occasionally in a condition of general collapse. If this primary pattern persisted through the whole strobila the uterine loop count would be a relatively stable and easily estimated taxonomic character. Unfortunately, the difficulty of loop counting is greatly increased by egg elimination—exhausted proglottids are almost impossible to use for loop counts—,and by distortion induced by egg pressure within the uterus.

In the primitive, long type of proglottis, the anterior loop count is approximately eight on either side of the middle line, the posterior count approximately three or four on either side. However, the later quadrate and linear proglottids show a reduction from these numbers, and their uterine loops, owing to the intrinsic pressure of the increasing number of eggs, aided by the extrinsic pressure of the muscular medullary margin, lose their parallel alignment and lie at an angle to the central area so that a rosette-like arrangement of the loops is produced. This is especially marked in the posterior uterine region. The bends of the loops become bloated with eggs, and in mounted preparations may give the appearance of a linear series of egg sacs on either side of the middle line. The two most anterior bends are particularly enlarged. Continued pressure brings about a corkscrew-like torsion of some of the anterior loops, or a sub-looping, so that in flattened preparations they may appear forked. Russian material shows this pseudo-forking to a marked degree; Manitoba material shows more of the torsion effect. If a fork be counted as two loops, then the loop counts of European material will appear greater than those of Manitoba material. The use of uterine loop counts as a taxonomic character is therefore not altogether satisfactory. The count is difficult to estimate with accuracy and the values found vary within relatively wide limits.

Anterior and posterior loop counts—each the mean of 20 consecutive proglottids—are given in Table II for material from various sources. It will be noted that the Manitoba human material agrees fairly well in having approximately 4.3 anterior loops and approximately two posterior loops on each side. The single Japanese specimen agreed on the whole with the Manitoba material. The Russian and Finnish material, owing to the prevalence of forked loops, showed higher mean counts of 5.6 for anterior and 2.5 for posterior loops. Material from dogs, both Russian and Manitoban, showed higher mean counts, the mean being 6.5 for anterior, 2.6 for posterior. For the material as a whole, excluding the Churchill dog material, the mean anterior count was 5.3, the posterior count 2.3.

The influence of secondary distortion upon the uterine loop counts is shown in Table III which gives the results of loop counts of 150 consecutive proglottids of Russian human material and 70 consecutive proglottids of Manitoba human material. To condense the table, only the means of each set of ten proglottids are given. It will be noted that in Russian material where secondary pseudo-forking is marked and the forks are counted as single loops, the uterine loop counts increase apparently from 3.3 : 3.2 in the anterior uterus, and 1.2 : 1.16 in the posterior uterus, to 5.5 : 5.2 : in the anterior uterus and 2.0 : 2.7 in the posterior uterus respectively, whereas in Manitoba material, where such pseudo-forking is rare and distortion produces chiefly

TABLE III

UTERINE LOOP COUNTS FOR CONSECUTIVE PROGLOTTIDS OF THE ONE STROBILA. THE VALUES REPRESENT MEANS OF SUCCESSIVE SETS OF TEN PROGLOTTIDS

Russian (human)		Manitoba (human)	
Anterior uterus	Posterior uterus	Anterior uterus	Posterior uterus
3.3 : 3.2	1.2 : 1.6	3.4 : 3.1	0.5 : 0.8
3.0 : 3.0	1.4 : 1.3	3.2 : 3.5	0.8 : 0.6
3.3 : 3.5	1.6 : 1.9	3.3 : 3.2	0.7 : 0.9
4.2 : 4.2	1.9 : 1.9	3.0 : 3.3	0.2 : 0.7
3.9 : 3.8	1.6 : 2.2	3.6 : 4.1	0.9 : 1.0
4.2 : 4.6	1.9 : 1.7	3.8 : 3.7	0.7 : 1.0
4.4 : 4.3	1.7 : 2.0	3.7 : 3.5	1.1 : 0.7
4.5 : 4.5	1.5 : 2.2		
4.3 : 4.5	1.9 : 2.4		
5.0 : 4.9	1.8 : 2.4		
5.2 : 4.6	1.5 : 2.5		
5.3 : 5.8	1.9 : 2.2		
5.2 : 5.5	2.3 : 2.0		
5.6 : 5.3	2.0 : 2.1		
5.5 : 5.3	2.0 : 2.7		

spiral twisting of the loops, the anterior uterine counts vary only between 3.4 : 3.1 and 3.7 : 3.5, the posterior uterine counts vary only between 0.5 : 0.8 and 0.7 : 1.0.

Conclusions

If we accept as valid criteria of specific distinction the characteristics of *Diphyllobothrium latum* discussed above, there does not appear to be sufficient difference between Manitoba human and canine material, Manitoba and Russian human material, Manitoba and Russian canine material, to suggest that more than one species is involved. There are however certain slight but constant differences between Manitoba and foreign material, and the Manitoba material may be defined as: *Diphyllobothrium* sp., probably *latum*, with scolex lanceolate to spatulate in shape but commonly clavate; with proglottids linear in human material but quadrate or even longitudinal in canine material; the dorso-ventral dimensions of the internal structures, expressed as percentages of the proglottid height are for the cuticle 0.34–2.8 (thickest in dogs), sub-cuticle 3.0–11.1, cortex 11.0–23.5, vitellaria 9.4–13.6, muscle zone 9.6–22.0 (thinnest in dogs), medulla 17.6–33.2, testes 14.0–24.6, cirrus sac (percentage of *maximum* proglottid height) 0.50–0.57. The position of the common genital pore, measured from its centre to the anterior proglottid margin, varies between 0.134–0.209 of the proglottid length. The uterine loop counts vary from 3.4–4.9 on either side, with a mean value of 4.3, for the anterior loops, and from 1.1–3.4 with a mean value of 2.0 for the posterior loops, in human material; in canine material the counts are 5.3–8.0 for the anterior loops, 1.3–3.9 for the posterior loops. Circumcloacal papillae absent or faintly visible in human material, prominent in canine material.

On the whole the Manitoba material shows somewhat closer resemblances to Japanese material than to European, but the limited amount of foreign material available for comparison precludes any definite conclusions to this effect.

Acknowledgments

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PLASMA LIPIDS IN ACTIVELY IMMUNIZED RABBITS[1]

By Eldon M. Boyd[2], J. H. Orr[3] and G. B. Reed[4]

Abstract

No statistically significant change was noted in the phospholipid and free cholesterol content of the plasma of 16 rabbits following a period of six weeks of active immunization against *Streptococcus viridans* as compared with 28 non-immunized controls.

Boyd (2) has shown that during fever and infection in man there occurs a decrease in the concentration of plasma lipids, to which reaction he has applied the term, lipopenia of fever. Investigating this reaction, Boyd, Orr and Reed (4) found that normal variations of body temperature were not accompanied by significant alterations in the percentage of plasma phospholipid and free cholesterol in healthy rabbits. From this and other evidence it appeared that variations in plasma lipid values during fever and infection were not due to the changes in body temperature *per se*. In the present study an attempt was made to determine whether the development of antigenic reactions is a causal factor in producing changes in lipid metabolism during fever and infection. Velluz (7) was unable to demonstrate any effect of immunization upon serum cholesterol, and Felton and Kauffmann (5) found no change in the alcohol-soluble material of blood, which may be taken as a rough index of the total lipid. Hyman (7), however, reported slight decreases in both phospholipid and free cholesterol in a brief report of work in which apparently little attention was given to variations which might have occurred in control animals.

The procedure in the present investigation was as follows. Sixteen healthy rabbits were actively immunized against a strain of *Streptococcus viridans* which had been isolated from a human case of endocarditis. The animals were injected on three successive days with increasing standardized doses of a vaccine prepared from the heat-killed organisms. This procedure was repeated 10 days later with larger doses and a third series of living organisms was given after a second 10-day interval. This procedure effectively produced active immunity. The agglutination titre of serum rose from an average of less than 1 in 10 before to 1 in 3200 after immunization, and the opsonic index rose from 1 to 3.2. None of the animals died or became seriously sick when the living organisms were injected. The organism was known to be actively pathogenic to rabbits because when injected into rabbits not previously immunized it invariably produced a marked infection and a high mortality rate.

Twenty-eight other rabbits were used as non-immunized controls. There occurred no significant change in the serum content of agglutinins or opsonins in the control group. In neither the control nor immunized group did any appreciable alteration in the rectal temperature occur.

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Blood for lipid analysis was taken after starving the animals for 15 hours, one specimen being obtained before and one after immunization, and at corresponding periods in the control animals. The samples of blood were heparinized and alcohol-ether extracts were prepared by the method of cold dilution (3). The phospholipid and free cholesterol content of these extracts were determined by the oxidative micromethods of Bloor as modified by Boyd (1).

The results obtained have been statistically summarized in Table I. Values reported include the average value before immunization, the average after, the average difference, and the standard deviation of the average difference calculated by a formula previously given (1). Presented in this manner, a difference to be significant should be at least twice its standard deviation. It may be seen from the table that the difference, neither with phospholipid nor with free cholesterol, was sufficiently great to satisfy the requirements of this criterion of significance. There was practically no change in the free cholesterol content before and after immunization, and the slight decrease in the means for phospholipid was not great enough to be statistically significant. Hence it may be concluded that following a period of six weeks of active immunization of rabbits with *Streptococcus viridans* there is no significant change in the serum content of phospholipid and free cholesterol. Since variations in the concentration of one lipid in plasma seldom occur without corresponding changes in the concentration of other lipids, it is probable that the immunization as performed would not have produced any changes in the concentration of any lipid in the plasma of rabbits. Keeping in mind that the experiments were performed only on one species and with one organism, they suggest that the changes in the concentration of plasma lipids during fever and infection are not due to the processes concerned with the production of active immunity.

TABLE I

EFFECT OF ACTIVE IMMUNIZATION UPON THE PHOSPHOLIPID AND FREE CHOLESTEROL CONTENT OF RABBIT PLASMA

(The results are expressed in mg. per 100 cc. of plasma.)

Group	No. of animals	Average phospholipid				Average free cholesterol			
		Before	After	Difference	Standard deviation of difference	Before	After	Difference	Standard deviation of difference
Immunized	16	71	54	−17	22	16	14	−2	12
Non-immunized	28	70	75	+ 5	23	19	19	0	7

The abstract given at the beginning of this paper may be taken as a summary of the results obtained.

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LARVAL AND ADULT STAGES OF BRITISH COLUMBIA ANOMURA[1]

BY JOSEPHINE F. L. HART[2]

Abstract

The external morphology of the adult stage of four species of Anomura (hermit crabs), *Upogebia pugettensis* (Dana), *Paguristes turgidus* (Stimpson), *Pagurus beringanus* (Benedict) and *Orthopagurus schmitti* (Stevens) is compared and the larval stages described. Emphasis is placed on the value of larval stages in clarifying our understanding of the taxonomy and interrelationships of the group. This study seems especially important in the Anomura because of the modifications of the adults which fit them for so specialized a mode of existence. It seems probable that some of the similarities exhibited by them are due more to convergence, as a result of similar mode of life, than to close affinity. This is illustrated by the superficial similarity of the adults of *Paguristes turgidus* and *Pagurus beringanus* and the dissimilarity of their larvae, as contrasted with apparent differences between the adult *Pagurus beringanus* and *Orthopagurus schmitti*, whose larvae are alike. By a comparison of the least modified appendages (the mouth parts) of the adults, the same relationship becomes apparent as was suggested by a study of the larvae. It is therefore concluded that by a comparison of both the larval and adult phases a clearer understanding of the affinities of the species under consideration is obtained.

Introduction

The classification of decapod Crustacea has been long a subject of controversy. One of the chief reasons for dispute is that the animals have been classified almost entirely by a consideration of adult characters. Thus it is to be expected that studies of the larval phases might clarify our understanding of the taxonomy of the group and perhaps suggest relationships not now appreciated. For instance, Lebour (23) has proposed a rearrangement of the classification of the British Brachyura based on a study of the larvae. A study of development would perhaps be even more illuminating in such a group as the Anomura, because of the modifications which fit them for so specialized a mode of existence. It is possible that some of the similarities which the adults exhibit are due to convergence as a result of a similar habit of life. Only ontogenetic studies can conclusively elucidate such a question.

The present study concerns four species of Anomura from British Columbia: *Upogebia pugettensis* (Dana), *Paguristes turgidus* (Stimpson), *Pagurus beringanus* (Benedict) and *Orthopagurus schmitti* (Stevens). The conclusions drawn as to the taxonomy of these forms are based on studies of the developmental stages as well as on the adult characters. Comparatively little

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information is available on the larval stages of Anomura and none of the forms considered here has been previously described. Except for characters used in identification, the morphology of the adults has received little attention.

Upogebia pugettensis is a symmetrical Thalassinid, not as greatly modified for its burrowing existence as many other members of the group, but with thin integument and lamellate pleopods, which are correlated with this mode of life. The other three species, however, do show modifications correlated with their life in spiral shells or in a straight tube. *Pagurus beringanus* and *Paguristes turgidus* live in gastropod shells and have twisted abdomens and asymmetrical tail-fans, while *Orthopagurus schmitti* lives in a worm tube and shows a lack of bilateral symmetry only in the difference in size of the chelae, and in the distribution of the pleopods. These species were not chosen haphazardly. *Paguristes turgidus* has a number of unusual structures in the adult and its larvae are quite unlike the described larvae of *Pagurus*. *Pagurus beringanus*, on the other hand, is typical in both larval and adult stages, and as its habitat is similar to *Paguristes*, seemed suitable for comparison. The third hermit crab, *Orthopagurus schmitti*, is of interest because of its greater abdominal symmetry and the form of its larvae. *Upogebia pugettensis* was chosen because it lives under somewhat the same conditions as *Orthopagurus* and exhibits a number of apparently primitive characters in all phases.

History of the Study of Classification

The classification of decapod Crustacea has been the subject of much discussion during the last hundred years and continues to be a difficult problem. In 1806, Latreille divided the order Decapoda into Macrura and Brachyura. The division was based on the type of abdomen:— in the first group, well developed and carried straight out behind, and in the second, turned forward under the thorax. This grouping, however, does not hold in the case of some families in which the abdomen is carried in the same manner as in the crabs or Brachyura, but which are obviously more closely allied to the long tailed Macrura. H. Milne-Edwards in 1834 introduced "Anomura", for a group intermediate between the other two and including the higher Macrura and lower Brachyura of Latreille's system. The members of this new group were those which had abdomens somewhat modified from the primitive macrurous type and still showed some remnants of a locomotive function and, with the exception of the Homolidae and Raninidae, retained paired appendages on the sixth segments. These two families are now usually placed in the Brachyura, but because of the type of larvae found in those species whose life history has been investigated, it seems probable that they will soon be moved again from the Brachyura. With the addition of some of the higher Macrura, the Anomura is now considered a natural group. In confirmation of this idea, Gurney (16) has found that the larvae of members of the group have at least one feature in common: the reduction in size of one pair of marginal setae on the telson.

Boas (6) pointed out that the divisions, Macrura, Anomura and Brachyura were not comparable nor were they of the same status taxonomically. His contention was that the Macrura consisted of a great variety of forms, some of which were more closely allied to members of the Brachyura or of the Anomura than they were to other members of their group. He therefore divided the order into the suborders Natantia or swimming forms, comprising the freely swimming shrimps, and the suborder Reptantia or creeping forms, including the Brachyura, the Anomura, the Thalassinidea, the Astacura, the Palinura and the deep-sea Eryonidea. Borradaile (10) adopted the main points of Boas' classification. His (9) most important change in this connection was the inclusion of the Thalassinidea (sand shrimps) with the tribe Anomura (hermit crabs), because of their resemblances to the primitive symmetrical hermit crabs. According to Borradaile, the only constant important differences are the presence in the latter of a pleurobranch on the last pereiopod, a suture across the telson, no epipodites on the legs, a reduced rostrum, subchelate fourth legs, and narrow uropods.

Benedict (3, 4) revised the nomenclature of the genera of the Paguridae and replaced *Eupagurus* with *Pagurus* and suggested *Pagurias* for those Pagurids described during the nineteenth century as *Pagurus*. These changes do not seem to have been universally adopted, but as recent American authors have used them in the revised form, *Pagurus* in this paper refers to those formerly called *Eupagurus*. This use, however, makes the names Pagurinae and Eupagurinae given in Borradaile's (10) classification untenable and therefore the subfamilies are referred to here as I and II.

Class *CRUSTACEA*
- Order DECAPODA.
  - Suborder *Reptantia.*
    - Tribe Anomura.
      - Superfamily Thalassinidea
        - Family Callianassidae
        - Subfamily Upogebiinae
          - *Upogebia pugettensis* (Dana)
      - Superfamily Paguridea
        - Family Paguridae
          - Subfamily I
            - *Paguristes turgidus* (Stimpson)
          - Subfamily II
            - *Pagurus beringanus* (Benedict)
            - *Orthopagurus schmitti* (Stevens)

Food

The feeding habits of all the species concerned in this paper are somewhat similar. McGinitie (26) says that *Upogebia pugettensis* feeds on detritus, which is caught by means of the "basket" formed by long hairs on the first two pairs of pereiopods through which water, containing particles of plant and animal remains, is forced by the action of the pleopods. The detritus is then cleaned off the "basket" with the third maxillipeds and conveyed by the other appendages to the mouth. If large particles of food are introduced, they are rejected by the animal.

No work has been done on the feeding habits of the hermit crabs of British Columbia, but as the habitat and modifications of the mouth parts are similar to those species that have been studied, it would seem safe to assume that they are alike in this respect. Orton (34, p. 920) states "*E. bernhardus* obtains food largely by its small claw and third maxillipeds, and may at certain periods of the year feed mainly on micro-organisms, and at others on macroscopic, but mainly tiny living organisms which have been disturbed and captured in the deposits on the sea bottom. Food can be obtained by the use of the third maxillipeds alone. The mouth parts of *E. bernhardus* are not adapted for rapid ingestion of the meats of animals in bulk, and the gastric mill is less strongly developed than in the carnivorous crabs: these facts, along with certain features of the gut, indicate morphological and anatomical modification correlated with the food and mode of feeding."

Orthopagurus schmitti, owing to its enforced stationary existence, is a plankton feeder, catching the organisms in the water by means of the action of its third maxillipeds, which are fringed with stiff bristles. The mandibles of all these species are comparatively feeble, indicating that the food does not require much mastication.

Material

The study of decapod Crustacea, on which the present paper is based, was commenced in the summer of 1928, when a collection of shore species was made in the vicinity of Victoria, British Columbia. The results of the study of this collection, together with some additional specimens obtained the following summer at Nanaimo, were published in 1930 (18). Specimens of adult Anomura, obtained while the writer was working at the Pacific Biological Station, Nanaimo, from 1929 to 1934, have been augmented by specimens from Victoria, and from points on the west coast of Vancouver Island, collected by Mr. E. G. Hart in 1934. Thus a considerable number of individuals of the species dealt with here were available for study.

Records of *Orthopagurus schmitti* have been published only by Stevens (40, 41), who described the species from Friday Harbor, Washington, and by the writer (18) from Nanaimo. *Upogebia pugettensis* is the only one of the species dealt with in which the morphology has been investigated except for those features used in the classification. The descriptions of the structure of the other three species are therefore given here for the first time.

Knowledge of the life history of decapods is scanty, especially concerning species from the Pacific coast of North America. From this area Berkeley (5) has published descriptions of some of the larval stages of five pandalid shrimps, and of five Hippolytidae (Berkeley (5) Needler (31)). Marukawa (27) described the larvae of the Anomuran King crab, *Paralithodes camtschatica* (Tilesius). All the larval stages of four species of true crabs were reared from the egg, and described by the writer (19). Thus the Anomuran larvae of British Columbia, with the exception cited above, are unknown.

The experimental rearing of Brachyura and Anomura was therefore started during the summer of 1930 (May until August) and continued during the same period of 1931, from January until July in 1933 and during July and August, 1934. Three species of hermit crabs were reared from the egg to the adolescent stages during 1931 and 1934 by the writer. In addition to these complete series, more than two dozen incomplete life histories, comprising about 75 post-embryonic stages, have been obtained by rearing from the egg. The culture methods have been described briefly (20, 21).

One soon becomes familiar with the characters of various groups and even of some of the genera, so that the stages can be readily distinguished in the plankton. The larval stages of allied forms are often very similar, and specific differences are difficult to determine. In a genus such as *Pagurus*, with some 15 species in British Columbia, careful and detailed comparison of the larvae is necessary, in order to determine specific features with any degree of certainty, and rearing from the egg is essential. The spawning season of most species is prolonged, so that the number of species to be found in the plankton at any time is great. The larvae are usually distinctively colored, but as all color is lost in preserving them, morphological differences must be relied upon. In the Anomura, the characters showing the greatest variation among species, and yet the least modification during the development and growth of the individual, are the carapace, the abdomen, the antennae and the telson.

Morphology of Adults

Upogebia pugettensis (Dana)

Upogebia pugettensis (Dana), the so-called mud-shrimp, is the first species dealt with here. There has been considerable controversy in the past about the position in the system of classification of the group to which this species belongs. The Thalassinidea are shrimp-like burrowing Reptantia. Correlated with their mode of life are certain modifications, such as a reduction in the number of gills, softening of the integument of the abdomen, and reduction in size of the pleura. It would seem probable that some of the characters in common within the group are due to convergence and not to close phylogenetic relationship. The Thalassinidea resemble the hermit crabs in some respects and the lobsters in others. Features common to both Thalassinidea and the lobsters, are the shape of the tail-fan, of the first leg and often of the second. They resemble the hermit crabs in the mode of concealment, in the thorn-like shape of the antennal scale (where this structure is present in the adult), in the freedom of the last thoracic sternite and the mode of carrying the last pair of legs. In view of these features, Borradaile (8) and Calman (12) put the family in the tribe Anomura, while Ortmann (32) and Bouvier (11) consider them to be more closely allied to the lobsters. Gurney (16), from a study of the larvae, feels that the resemblances to the Anomura are greater in this phase. He also points out that the Thalassinidea is not a homogeneous group, and that with further knowledge it will be split into at least two series. We are, however, concerned here only with one species of the subfamily Upogebiinae and its resemblance to the hermit crabs, and in this connection the Anomuran affinities are of greatest interest.

Stevens (42) has ably described and figured this species and made ecological notes. The ecology has also been studied by McGinitie (26). The papers of these two writers have been most useful in the discussion and comparison of the species dealt with in this paper.

It will perhaps suffice to mention that *Upogebia pugettensis* (Dana) is macrurous in shape, often reaching 90 mm. in length of body, and dwells in burrows, about 25 mm. in diameter, tunnelled in sandy mud or gravel by means of the animal's walking legs and tail-fan. The species occurs commonly on tide flats from Southern Alaska to Lower California.

Paguristes turgidus **(Stimpson)**

Paguristes turgidus (Stimpson) is a hermit crab inhabiting fairly deep water (to 254 fathoms) from British Columbia to Southern California. It usually occurs on a rocky bottom and in considerable abundance. The shell commonly occupied is the moon shell, *Polinices*, and there is a tendency for the individual to choose a shell larger than necessary, so that it can withdraw itself completely within its shell and can be reached only by breaking the shell. Although this is the only species of the genus recorded from British Columbia, other species are found in California and in nearly all tropical and semi-tropical regions.

P. turgidus may have a carapace of about 25 mm. in length, with a total body length of 75 mm. It is an interesting form because the chelae are subequal, the left being the larger; paired appendages in addition to the uropods are present on the abdomen and there is an egg pouch in the female. The animal is in general a deep red, toned down in color by tan hirsuteness and the brown bases of spines. The antennules and eyestalks are striped longitudinally with red and white. The abdomen is yellow, except in the mature female, where the ova show scarlet through the thin integument. The pleopods are pink and the uropods red with yellow setae.

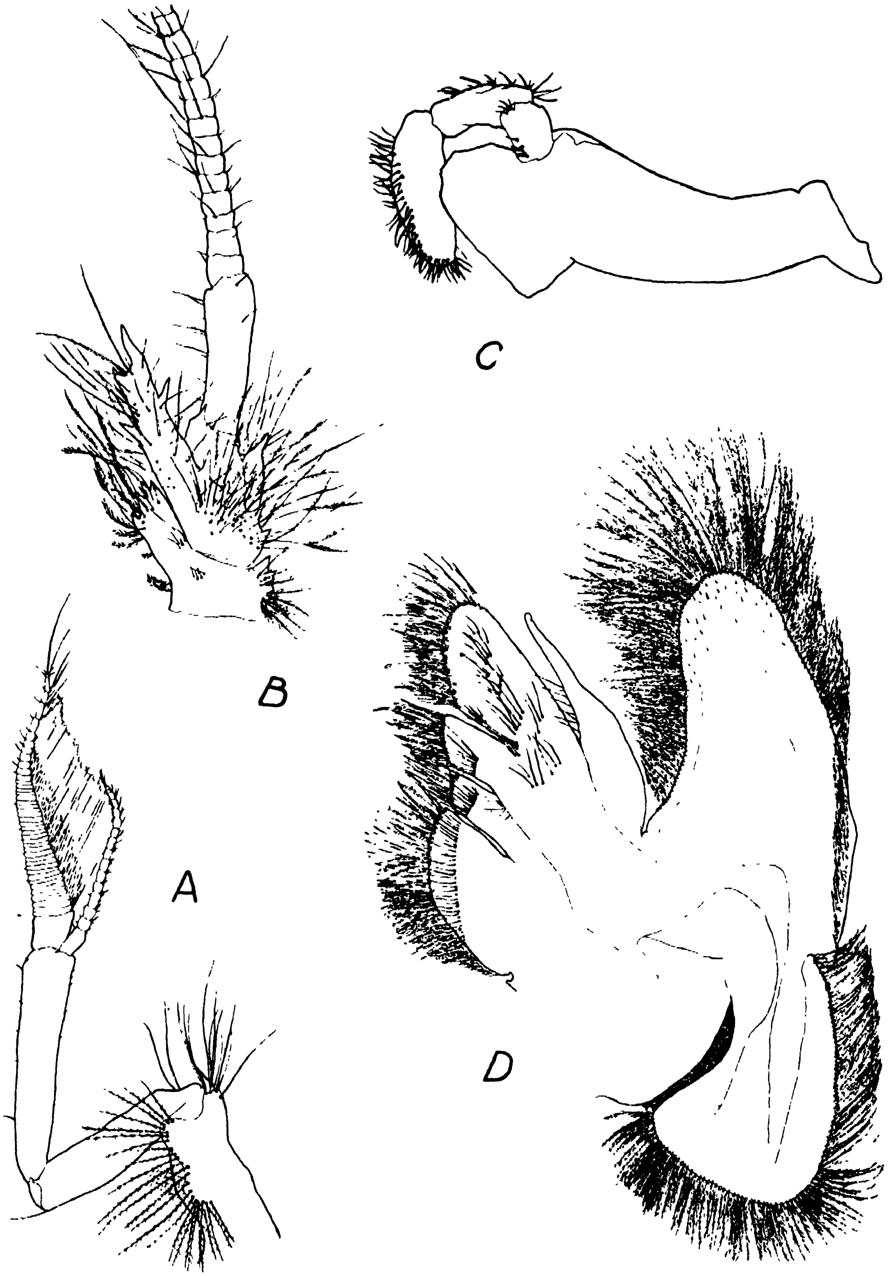


FIG. 1. *Paguristes turgidus (Stimpson). Male. A. Antennule. B. Antenna. C. Mandible. D. Maxilla.*

Female (Plate I, Fig. 1; Fig. 3, A-C)

Paguristes turgidus (Plate I, Fig. 1) is a stout species: hairy, and with dark-tipped spines on the subequal chelae and walking legs. Most of the hairs are plumose. Carapace thinly calcified, especially the posterior part. Rostrum and lateral spines subequal: small and sharp-pointed. Abdomen large, dextrally twisted, with tergum and sternum of first segment calcified; as is area around pleopods on left side and corresponding region of right side, of second to fifth segments. Sixth segment and telson also calcified. Some indication of calcification along ventral margin of outgrowth (which serves as a partial egg pouch) found on posterior part of fourth segment on left side. Telson (Fig. 3, A) asymmetrical, left side much larger than right, with sutures cutting off triangular postero-lateral portions: posterior margins toothed. Eyestalks long, cylindrical, and scales cut into a number of deep teeth.

Antennule rather flattened laterally; the first segment of peduncle with three large spines, the second and third segments narrower than first. Inner flagellum large, multiarticulate, and nearly twice as long as narrow outer flagellum. Antenna has short coxopodite; basipodite armed with a large spinous protuberance, about half the size of spined scale (exopodite): first segment of endopodite has a large spine, second a smaller one and third smooth and equal to sum of lengths of other two; flagellum comparatively short; stout and hairy.

Right mandible with a somewhat triangular incisor and a rounded molar; small basipodite; first segment of endopodite with a few setae and second with numerous bristles. Left mandible similar, except that cutting surface is rounded, with a few flat teeth.

Maxillule with endite of coxopodite broad and that of basipodite narrower and longer: endopodite rather large, with bristles on tip and large lateral and posterior extension (second segment); exite (epipodite ?) large. Maxilla has large endites, heavily bristled: a fair-sized endopodite, tapering towards tip, with sparse lateral bristles; scaphognathite large with dense brush of setae on margin, and a fold indicating fusion of exopodite and epipodite.

First maxilliped with a thick triangular coxopodite, and a flat basipodite produced distally, with stout bristles on inner margin. Endopodite composed of a single curved segment; proximal portion of exopodite swollen, heavily armed with hairs, and bearing a narrow segment with flagellum of a few poorly defined segments: and epipodite large and lying against exopodite. Second maxilliped of usual type, with endopodite of five segments and a well developed exopodite with flagellum. Third maxilliped stout with bases of appendage closely approximated: endopodite armed with a row of heavy spines on ventro-medial margin of merus, other segments with stiff bristles; exopodite large and flagellate. Two arthrobranchs: phyllobranchiate with rather elongated leaves.

First pereiopod chelate, left subequal to right; rather short, stout and heavily armed with spines and plumose hairs, except on medial surface and

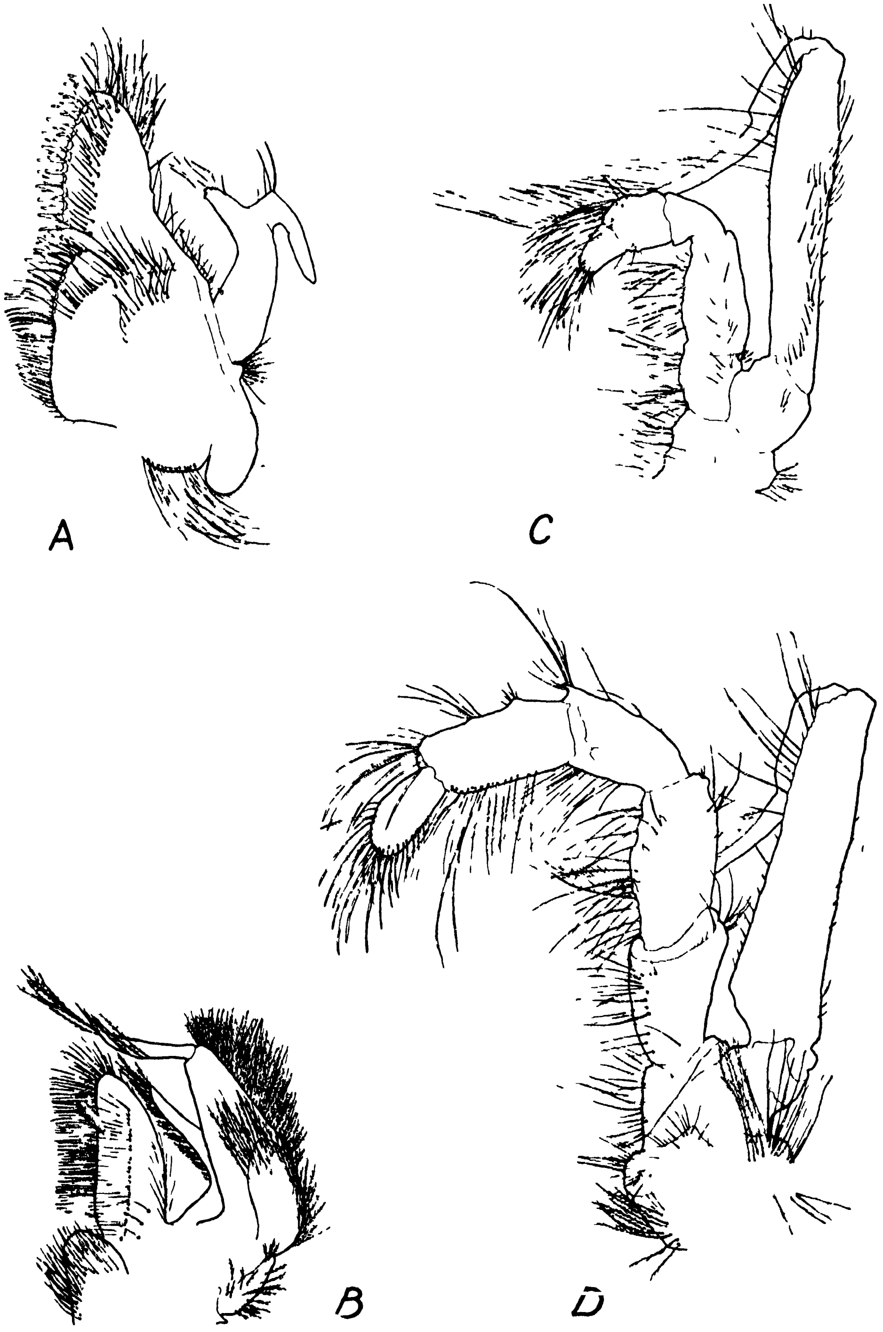


FIG. 2. *Paguristes turgidus (Stimpson). Male. A. Maxillule. B. First maxilliped. C. Second maxilliped. D. Third maxilliped.*

outer side of merus; tips of chela strongly sclerotized. Two arthrobranchs. Second and third pereiopods well developed, elongated and armed with spines and plumose hairs. Each has two arthrobranchs and one pleurobranch. Fourth pereiopod small, with long hairs and suctorial setae on distal ventral surface of propodus. It is simple, not subchelate. Two arthrobranchs and one pleurobranch. Fifth pereiopod, which is carried folded up posterior and dorsal to carapace, also small, with bunches of hairs and definitely chelate (broad flat thumb and movable finger) with suctorial setae. No gills.

First pleopod (Fig. 3, B) small, with fused joint. Distal part probably endopoditic in origin, because in *Upogebia* a similar pleopod carries eggs, and these are borne only by endopodites. Few marginal setae. Second pleopod (Fig. 3, C), as well as third, fourth, and fifth, borne only on left side. Second made up of protopodite, rather broad distally, and subequal endopodite and exopodite. Third similar, except that exopodite larger than endopodite. Fourth has an even larger exopodite than third. Fifth pleopod has no endopodite, but exopodite well developed. Uropods or appendages of sixth segment (Fig. 3, A) unequal in size: that of the left side much larger than right, and both with exopodites larger than endopodites. The flattened posterior surfaces bear numerous suctorial setae.

Male (Plate I, Fig. 2; Fig. 1; Fig. 2; Fig. 3, D-E)

Male of *Paguristes turgidus* (Pl. I, Fig. 2) differs from female mainly in armature of abdomen and its appendages. Abdomen of male more strongly calcified than that of female. First segment is similar to that of female. Tergum and part of sternum of second calcified, as is a large part of tergum of third segment, which is armed with soft hairs posteriorly. Fourth and fifth like third, but to a lesser degree, while sixth has tergum strongly calcified.

Paired abdominal appendages of first and second segments highly specialized for transference of spermatophores to body of female. Paired appendages (Fig. 3, D) of first segment lie fairly close together; each is a short, stout, uniramous limb of two segments. Protopodite margined on inner edge with stout setae and endopodite(?) hollowed out, twisted and armed with stout bristles and teeth to form upper half of tube. Second pair of pleopods (Fig. 3, E) longer and their bases farther apart with protopodite unarmed and distal half of endopodite(?) grooved to fit over that of first appendage. Second pair of pleopods covered with coarse bristles distally. Remaining pleopods (present on left side only) all lack endopodites (with one exception, a small endopodite on third pleopod of large male figured). In lacking endopodites, male differs from female of species and from males of *Pagurus* and *Orthopagurus*. Pleopods of third and fifth segments subequal in length and that of fourth somewhat longer. They all bear tufts of setae on protopodite, and exopodites are margined with numerous long plumose setae.

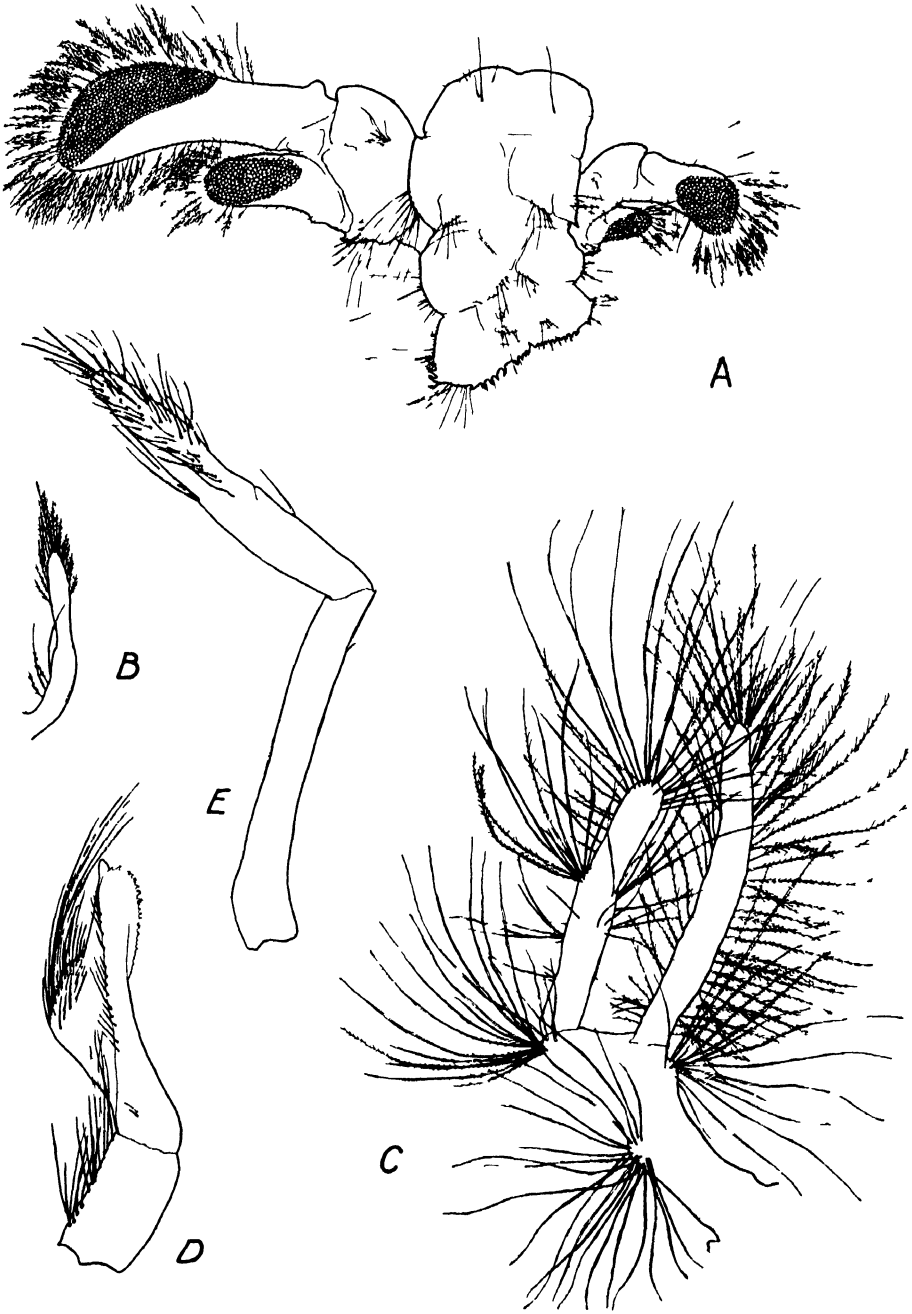


Fig 3 *Paguristes turgidus (Stimpson) A-C, Female A Telson and uropods B. First pleopod. C. Second pleopod. D, E, Male. D. First pleopod E Second pleopod.*

Pagurus beringanus (Benedict)

Pagurus beringanus (Benedict) is a typical hermit crab, with large right claw and asymmetrical abdomen with paired appendages on the sixth segment only. In the Nanaimo region, *P. beringanus* is commonly taken with *Paguristes turgidus*, and for this reason is used here. It is, however, a shallow water species, not having yet been obtained from water more than 40 fathoms deep. In the Nanaimo area, the adults are not found near the shore at low tide, but they are common on all rocky shores in the Straits of Juan de Fuca. The lower temperature of the water in the latter area may account for this difference in distribution.

P. beringanus usually occupies the empty shells of the whelk, *Thais* and, like Paguristes, chooses large ones. Frequently there is a break in the shell near the tip, and a small individual of the same species lives in the upper part, with a large one in the lower. The coloring is brilliant: gray-green chelipeds, except for a white band near the tips, with orange granules and spines; the pereiopods green and white, spotted with rust, which is concentrated into a band at each joint; and the remainder of the body flecked with scarlet and brown; mainly scarlet on the branchial region of the carapace and on the abdomen. The eyestalks and antennae are brown.

Female (Plate I, Fig. 3; Fig. 4, A-C)

Pagurus beringanus (Plate I, Fig. 3) is quite a stout species, with a fair number of fine hairs (none plumose). Chelipeds armed with short spines and spiny granules. Carapace may reach a length of 20 mm. and body about 50 mm. Carapace fairly well calcified anteriorly, but thin and soft posteriorly. Rostrum and lateral teeth very obtuse. Abdomen with calcified area on tergum of first segment; two lateral, elongated patches on second and third segments, and small round areas on fourth and fifth. No brood pouch. Sixth segment and telson (Fig. 4, A) similar to those of *Paguristes turgidus*. Eyestalks long, but scale small and triangular and not cut into teeth.

Antennule like that of *Paguristes* except that there are two rows of long bristles instead of scattered short setae on inner flagellum. Antenna is a narrower appendage with spines on basipodite, with a narrow sickle-shaped exopodite and a longer flagellum, with very short bristles between articulations.

Mandibles each have three blunt teeth and the separation between basipodite and endopodite is not clearly defined. Endites of maxillule not as well developed as those of *Paguristes;* endopodite differs in having only one bristle on inner side of flattened apex and a small knob on outer corner. Exite much smaller. Maxilla very like that of *Paguristes* except that there are bristles at tip of endopodite, and no suture which might indicate that the scaphognathite is made up of two parts, as is seen in the other form.

First maxilliped (Fig. 4, C) much smaller and less twisted than that of *Paguristes.* No epipodite. Second maxilliped similar in the two forms but

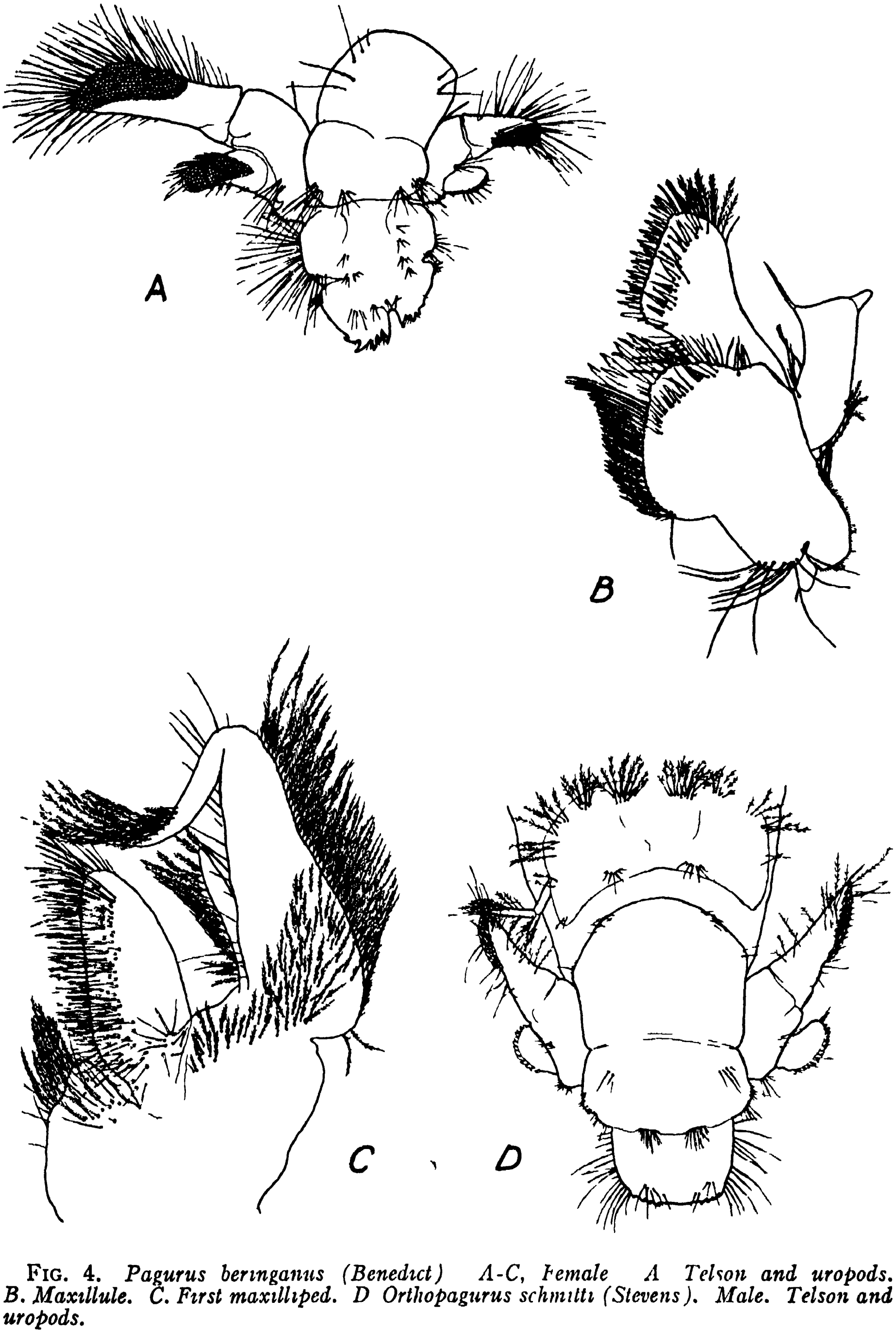


FIG. 4. *Pagurus beringanus (Benedict) A-C, Female A Telson and uropods. B. Maxillule. C. First maxilliped. D Orthopagurus schmitti (Stevens). Male. Telson and uropods.*

flagellum slightly shorter. Third maxilliped is smaller than that of *Paguristes*, but like that species has a row of teeth on inner edge of ischium, and there are a larger number of comb setae on dactylus and propodus, in fact the whole endopodite is more densely armed with bristles. Coxae separated. Two arthrobranchs on each appendage.

Right cheliped much larger than left, and both with rather short thumbs and fixed fingers. Two large tubercles present on ventro-distal part of merus of right one. Two arthrobranchs. Next two pereiopods long, with no pleurobranchs. Fourth very small, about $\frac{2}{3}$ that of *Paguristes*, subchelate with tuberculate edge on short propodus. Two arthrobranchs and one pleurobranch. Fifth leg also small, chelate, although not as clearly so as in *Paguristes*.

No pleopods on first segment of abdomen; and those of second, third and fourth segments differ from *Paguristes*, in that the bases of exopodite and endopodite are close together, and the end of protopodite narrow. The two parts are more nearly equal in size. Fifth pleopod has a short endopodite. Uropods similar to those of *Paguristes*.

Male

Integument of abdomen of male is like that of female in degree of calcification. No pleopods on either side of first and second segments, and only on left side of third, fourth and fifth. Pleopods have a large exopodite and a small endopodite.

Orthopagurus schmitti (Stevens)

Orthopagurus schmitti (Stevens) is a small hermit crab (up to 40 mm. in length) inhabiting empty worm tubes of two species of *Serpula* and of *Sabellaria cementarium* Moore. The species is of interest here because the abdomen is straight, not coiled as in *Pagurus* and *Paguristes*, and the telson and uropods are symmetrical, but the other abdominal appendages are on the left side only as in *Pagurus*. It occurs in shallow water (to a depth of about 100 fathoms) and is obtained by dredging up the rocks to which the worm tubes are attached. At minus tides, some of these rocks are exposed at Horswell point, but in other places dredging is necessary. It is quite common locally, but has been recorded only from the Friday Harbor and Nanaimo regions.

O. schmitti is somewhat pubescent, with brown hairs. The coloring is bright: pink or white with orange-brown bands and patches.

Female and Male (Plate I, Fig. 4; Fig. 4, D)

Carapace with a hard smooth cephalic portion, and the remainder very lightly calcified. Rostrum of a fair size, but lateral teeth small. Eyestalks fairly stout, pear-shaped, and a small triangular scale. First segment of abdomen calcified, as are small patches on either side of dorsal part of second, third and fourth segments. No columellar prominence. Fifth segment with part of tergum calcified, showing indication of an overlapping fold posteriorly and apparently used to assist in retaining a hold in tube. Sixth segment of abdomen (Fig. 4, D) with tergum well calcified and divided very definitely by a deep groove. Telson (Fig. 4, D) quite symmetrical, with rounded lateral margins and a few shallow teeth on posterior straight margin.

Antennule like that of *Paguristes turgidus* except that there are no bristles on peduncle. Antenna like that of *Pagurus*, except that setae between articulations of flagellum longer, but shorter proportionately than those of *Paguristes.*

Mandibles, maxillae, maxillipeds and first three pairs of pereiopods resemble those of *Pagurus beringanus.* Fourth pereiopod smaller than fifth, subchelate and flattened distally. Fifth does not reach carpus of third, and is chelate. Pleopods present on left side of second to fifth segments. Endopodite and exopodite subequal except for fifth, where endopodite small; protopodite narrow. Uropods (Fig. 4, D) subequal and similar, with large exopodite and small endopodite.

Pleopods of male present only on third to fifth segments.

Comparative Morphology of the Genera

The problem is to determine those characters which are not the results of modifications due to a similar mode of life (convergence) but which remain comparatively unaffected by a change of habitat and also to find those that are vestigial.

The four species dealt with here have a somewhat comparable habitat and they exhibit certain modifications in common. They all live protected by substances not secreted by themselves, *i.e.*, hollow objects: *Upogebia* in its sand burrow, *Paguristes* and *Pagurus* in gastropod shells and *Orthopagurus* in calcareous or stony worm tubes. Thin integument is found in each, especially on the carapace and abdomen. This seems to be a character associated with life in a protected spot.

Paguristes resembles *Upogebia* in the adult phase in a number of morphological characters. The rostrum of both species is small and the surface of the carapace posterior to it, roughened. In *Upogebia,* according to McGinitie (26), this serves to hold the animal in position against the top of the burrow while the pleopods fan the water for food and for aeration. In *Paguristes,* it probably assists the animal to retain a hold in its shell and to resist efforts to dislodge it. The flagella of both species are rather short and hairy and although the adult *Upogebia* has but a vestigial scale on the antenna, some allied forms have it well developed. The endopodites of the maxillules are two-jointed and the exite (epipodite?) large. There are indications that the scaphognathite of the maxilla of both species is made up of two parts (exopodite and epipodite). The bases of the third maxillipeds are approximated. The first pair of pereiopods are alike (chelate) and subequal; the fourth pair are simple (neither chelate nor subchelate) and the fifth is chelate in *Paguristes* and often in *Upogebia.* In the females of both species, the pleopods of the first segment are small, paired and uniramous. The males have paired pleopods on the second segment, but these are modified for copulation in *Paguristes* only. The pleopods in *Upogebia* serve as a means of creating a powerful current of water through the burrow and incidentally drawing in a supply of food, while those of *Paguristes* need only occasionally stir the water within the shell. The females of both species carry the eggs on the setae of the endopodites.

In all these characters except the last, *Paguristes* differs from *Pagurus* and *Orthopagurus*, but resembles them in its mode of life and many of the modifications that fit it for such an existence, particularly the general body shape. The symmetry or lack of symmetry of the appendages does not coincide in the three species.

The carapace of *Paguristes* has the same general shape as that of *Pagurus*, but the distribution of the hard parts (calcified chitin) and of the grooves is different. The abdomen is twisted like that of *Pagurus* (of the same habitat), but shows more vestiges of segmentation. It differs, as does *Pagurus* in this feature, from *Orthopagurus*, which has a symmetrical abdomen suited for life in straight tubes. The antennules, antennal scale, mandibles and second and third maxillipeds, are similar in the three species, but are of the unspecialized decapod type, with the exception of the antennules, which are more like those of the Brachyura, than the more primitive type found in *Upogebia*. The first pereiopod is so twisted that the chelae open horizontally and the axis of the joint between the carpus and propodus is such that an "operculum" may be formed by the chelae, thus preventing intruders from entering. *Upogebia* uses the tail-fan for blocking the entrance to its burrow. The fourth and fifth legs are reduced in size, but as there is a tendency in all Anomura for the differentiation of the fifth legs, and their specialization for cleansing purposes, only the reduction in size of the fourth legs is of interest here. In *Pagurus* and *Orthopagurus* this reduction is greater than in *Paguistes* (30% and 40% of the third legs). *Paguristes* resembles *Pagurus* and *Orthopagurus* in the absence of pleopods on the right side of the second to the fifth segments of the female and of the third to the fifth of the male. The telson and uropods are asymmetrical in *Paguristes* and *Pagurus*, but not in *Orthopagurus*.

Unique Features

Paguristes turgidus differs from *Upogebia*, *Pagurus* and *Orthopagurus* in several important characters:

1. The grooves of the carapace.
2. The presence of a greater number of gills. There are ten arthrobranchs and three pleurobranchs on each side, as compared with ten arthrobranchs only in *Upogebia*, and ten arthrobranchs and one pleurobranch in *Pagurus* and *Orthopagurus*.
3. The presence of paired appendages specialized for copulation on the first and second segments of the abdomen of the male.

4. The presence of an outgrowth or egg pouch on the left side of the fourth segment of the abdomen of the female. This structure is found only in one other genus, *Paguropsis*, which according to Alcock (1) is closely allied to *Paguristes*, and differs only in having a straight abdomen, symmetrical telson and uropods, cheliform fourth legs and unpaired appendages and egg pouch indifferently on the right or left side of the abdomen.

Orthopagurus schmitti differs from the other three species in the partial restoration of the tergum of the fifth segment of the abdomen and in the symmetrical uropods adapted for holding the body within a hollow worm tube.

History of the Study of Development

Until about a hundred years ago it was believed that all decapod crustacea developed directly, in other words, with no metamorphosis. When Thompson (43) hatched the eggs of *Cancer pagurus* and obtained the zoeae, his report on these findings was not credited by many of the scientific men of the times. Westwood insisted that "no exception occurs to the general law of development in the Crustacea; namely that they undergo no change of form sufficiently marked to warrant the application to them of the term metamorphosis" (2, p. xliii). This idea was deeply implanted in the minds of contemporary workers largely because of the extensive work of Rathke on the development of the river crayfish, *Astacus fluviatilis*, in which, because of the life of the adult, planktonic larval stages would not be feasible. The confusion at this time was increased by observation of female crab abdomens from the tropics, to which fully developed and recently hatched young were attached. These were believed to be land crabs (13) which naturally would be governed by the same restrictions as the crayfish.

Thompson (44) described the larvae of 15 decapods including *Pagurus*. The post-larval hermit crabs were first described as adult animals under the generic name of Glaucothoë. Müller (30) surmised their true relationship to the adults.

Culture Methods

General methods for culturing both adult and larval Anomura have been given previously (20, 21). Therefore only a few additional notes are added here.

The study of the larval development of any marine form is fraught with difficulties, which are augmented in a locality where the life histories of few of the species have been worked out. Bovard and Osterid (7) published a list of those animals from which embryological material may be obtained during the months of June, July and August at Friday Harbor, Washington. This list is useful for work in the immediate vicinity of Victoria but is quite misleading for the Nanaimo district. In the latter region, although it is nearly 100 miles north of Friday Harbor, or Victoria, hydrographical conditions are more temperate, and many species spawn weeks, or even months, earlier in the season.

Few papers give any information on the spawning of Pacific coast decapods, but some record ovigerous females, and this information is sometimes of value in the case of forms that do not carry their eggs for a long period, and those that have a definite spawning season. The recording of "ovigerous" females does not take into account the degree of development of the eggs. It has thus been necessary for the present author to make her own records of the egg-carrying and of the spawning periods of the hermit crabs of British Columbia. The approximate state of development of the eggs has also been kept.

The period of incubation of the eggs differs greatly, but seems to vary in most cases directly with the size of the adult. It was found difficult to keep berried females of *Upogebia pugettensis* alive in aquaria, and thus to determine approximately the length of time the eggs are carried. McGinitie (26) found it necessary to provide the "shrimps" with tubes to live in. It is also difficult to induce *Paguristes turgidus* to hatch its eggs in the laboratory. The female will cast off a few eggs each day so that unless they are nearly ready to hatch when obtained, they will all be shed before hatching time. If a few still remain, they are generally delicate and die without casting the prezoeal cuticle.

A minimum of exposure to light seems to be preferred by most, but except for direct sunlight, fairly bright light does not appear to be especially detrimental. They are, however, more active at night, and usually the eggs hatch at this time. As low a temperature as is practicable is advisable (15–20° C.), but the majority seem able to withstand a surprising range.

Hermit crabs as a whole do not thrive without shells and as it is necessary to be able to observe the state of the eggs from time to time, the most satisfactory method is to break a window in the shell, through which the eggs may be seen, even though the animal has retired into her shell.

The egg yolk of *Upogebia* and thus the egg mass, is a bright yellow when freshly extruded, but with the absorption of the yolk and the development of the eye pigment, the mass changes to a brownish color. The egg mass of *Paguristes turgidus* is at first a bright coral-red, changing with development to yellow. The egg mass of *Pagurus beringanus* changes from brownish black to gray. The eggs of *Orthopagurus schmitti* have not yet been seen.

In the jars containing larvae, the prevention of too abundant growth of diatoms and protozoa was accomplished by moving all the larvae, by means of a large-bore pipette, as frequently as necessary to a fresh jar of sea water and food material. The sea water used was not filtered, but care was taken not to include such forms as amphipods and jellyfish, as these will clean out a jar of larvae in an extremely short time. Unless the inner surfaces of the jars were cleaned carefully, worms and barnacles were found to have settled down and grown large enough to be a menace to the larvae.

The rearing of the larvae of hermit crabs is a long and tedious process, and the mortality is extremely high. Only by repeated attempts and by the use of a large number of individuals, may a few be brought beyond the early stages. Failure has been complete and consistent in many species, the larvae never becoming robust enough to cast off the last embryonic cuticle. However, if sufficient berried females are available, the first larval stage may be obtained occasionally, but the emergence of second stage larvae from first is the crucial point. The post-larvae are comparatively easy to keep if they are healthy when they reach this stage. The adolescent stages of those forms that will eat minced clam muscle thrive as long as they are not crowded or underfed, under which circumstances cannibalism occurs.

Larval Development

SUBFAMILY UPOGEBIINAE

The larval stages of European species of *Upogebia* and *Gebiopsis* have been described by various authors. Sars (38) studied a Norwegian form, Cano (14) a Mediterranean, and Webb (46) the British species. Doubt has been expressed as to the correctness of identification of these species, but since the larvae are certainly the young of these closely allied genera, the exact species is quite immaterial for comparison with the larvae of *Upogebia pugettensis* (Dana), which is found only on the Pacific coast of North America. Gurney (16) and Menon (28) have described larvae from the plankton of New Zealand and Indian waters. *U. danai* from New Zealand and *U. pugettensis* are quite similar, while the *Upogebia* (?) sp. from India shows interesting intergradations between *Upogebia* and *Paguristes* in the larval stages, a subject which will be dealt with later. Gurney (17) describes a *Upogebia* from the Red Sea in which there is no metamorphosis; the first free living form resembles the post-larva of other species.

Gurney (16) gives the characters which distinguish the larvae of Upogebiinae and with these *U. pugettensis* agrees, except for one character, namely, the presence of a distinct unarmed palp on the mandible of the second and third larval stages. The first larval stage of this species is somewhat more advanced than *U. danai* as the pleopods are quite elongate, and can hardly be described as "minute knobs", as in the latter. The second stage is similar in having six pairs of thoracic appendages with functional exopodites, but differs by the presence of a mandibular palp. The third stage also differs from that of *U. danai* and the European forms, in the shape of the telson, which is more triangular and has the posterior margin sloping slightly inwards from the lateral to the median spines. The fourth pair of spines are not strongly developed and those lateral to these are on a level with them, not on a sloping margin. There is no lateral spine on the exopodite of the uropod as is seen in other described *Upogebia*.

Upogebia pugettensis (Dana) (Figs. 5 and 6)

At Friday Harbor, Washington, females of *Upogebia pugettensis* (Dana), bearing eggs, were reported by Stevens (42) in December, February, and in the late spring and early summer. They have been taken at Departure bay, from January until April, and the larvae occur in the plankton from February until the end of May. The egg mass is bright yellow when freshly extruded and changes to brown when ready to hatch.

There are three larval stages before the post-larval, corresponding in this respect to some species of the genus as described by Webb (46), but the larvae of this species hatch in a slightly more advanced state of development than do the European forms. This was also found to be the case in some of the Pandalids of British Columbia (5).

First larva about 3.7 mm. in length, measured from tip of rostrum to posterior margin of telson. Transparent, with a blue tinge to body, and liver showing through carapace as an olive green organ. Yellow around eyestalks, and a red chromatophore near tip and another on proximal part of antennule. Red pigment on carapace anterior to gastric mill, as well as on proximal part of first abdominal segment and on median part of telson (two chromatophores). A branching red chromatophore on basal part of non-functional third maxilliped.

Larva (Fig. 5, A, B) of the same type as those described by Webb (46). Carapace with rounded edges posteriorly and small, sharp-pointed rostrum reaching about one-third length of antennules. Abdomen composed of five segments and deltoid telson. Posterior margin of telson indented medially with a spine, a fine hair and five plumose setae on each half. Segments of abdomen smoothly rounded posteriorly, without dorsal or lateral spines.

Tubular antennule (Fig. 6, A) quite long, with several aesthetes on inner margin and terminally, and a long plumose seta a short distance from tip. Antenna (Fig. 6, B) large: protopodite with a single spinous projection ventrally, endopodite tubular with three long plumose setae terminally, and broad exopodite terminating in a stout spine and bearing eight long and one short plumose setae on inner margin.

Mandibles (Fig. 6, C) without incisor processes, and molar surfaces cut into a number of small teeth. Maxillule (Fig. 6, D) with wide clearly defined endites on protopodite, each bearing eight spines, and the two-jointed endopodite with two plumose setae on distal portion of internal edge of first segment and six on second (four terminal and two sub-terminal). Lamellate maxilla (Fig. 6, E) with endites of protopodite bilobed: coxopodite bearing ten and four setae and basipodite five and six. Endopodite with five plumose setae, but is not clearly separated from protopodite like Brachyuran zoea, and scaphognathite narrow, with about 11 plumose hairs on margin.

First maxilliped (Fig. 6, F) provided ventrally with a number of bristles, both plumose and simple: two on coxopodite and about 11 on basipodite. Endopodite five-jointed, and segments usually with three, three, one, two and five bristles respectively on ventral distal part of segments, and fine hairs on the second, third and fifth segments dorsally. Four swimming setae on exopodite. No setae on coxa and basis of second maxilliped (Fig. 6, G) but four swimming setae on exopodite. Endopodite four-jointed, with third segment

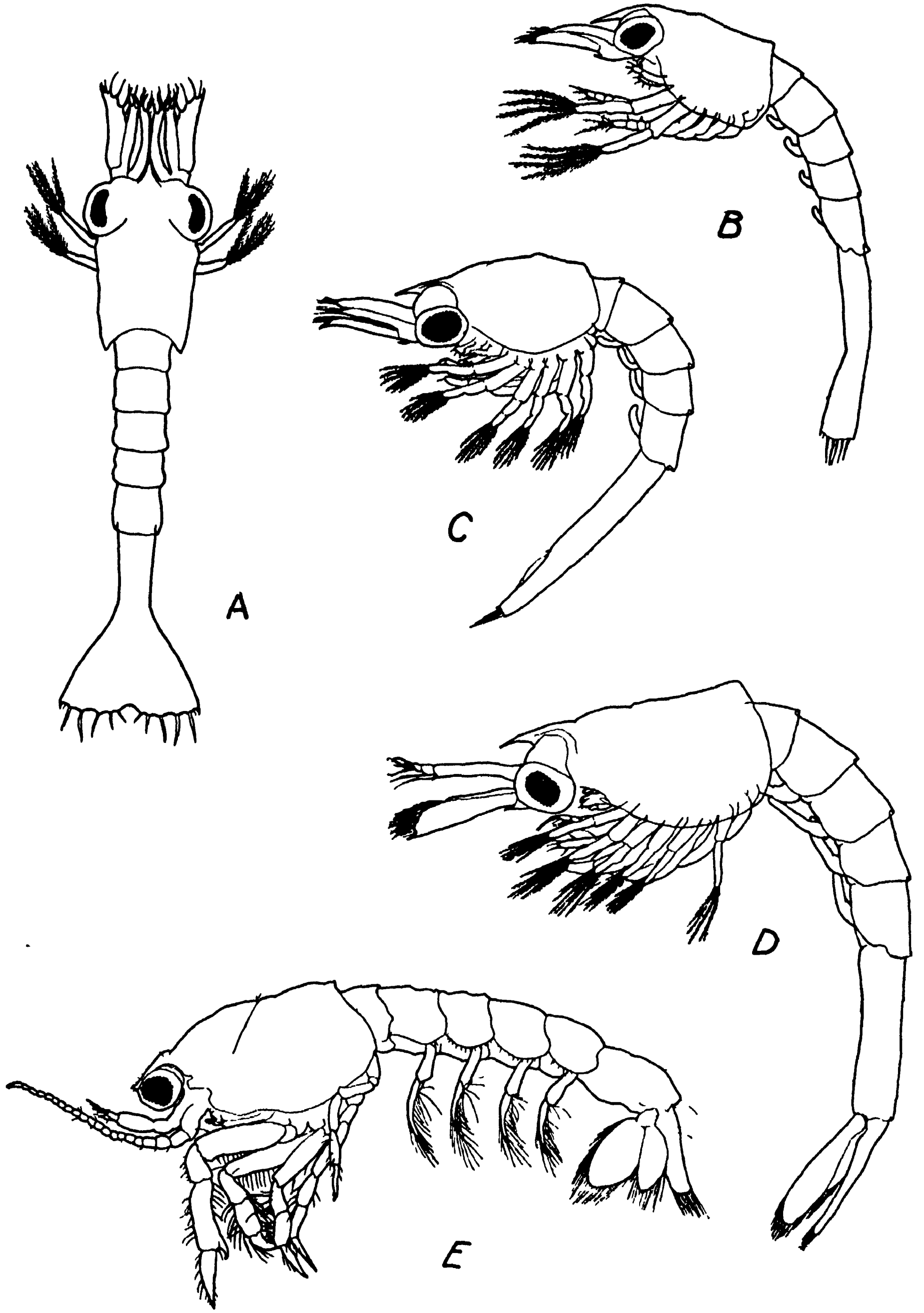


FIG. 5. *Upogebia pugettensis (Dana). A. First larval stage, dorsal view. B. First larval stage, lateral view. C. Second larval stage, lateral view. D. Third larval stage, lateral view. E. Post-larval stage, lateral view.*

equal in length to the other three, and segments bear one, one, two and three setae respectively. Large, non-functional exopodites present on rudimentary third maxilliped and on first three pairs of pereiopods. Endopodites on all these anlagen well developed. Four pairs of pleopods will be developed, indicated in first stage by knobs on abdomen.

Second Stage (Fig. 5, C; Fig. 6, H-L). Second larva (Fig. 5, C) about 4.4 mm. in length and similar in color to first. Sixth abdominal segment not yet separated from telson (Fig. 6, H) which is similar in shape to that of first stage, but has an extra pair of plumose setae medially and a small median tooth on posterior margin. Fourth pair of setae now considerably longer and stouter than others. Uropods show clearly through integument but still fully enclosed within telson membrane.

Antennule (Fig. 6, I) larger and tip cut into two knobs: anlagen of flagella of adult. Outer is larger and bears aesthetes, and more slender inner knob sharp-pointed, and bears fine setae. Several plumose setae on inner and a tuft of fine hairs on outer margin of peduncle. Antenna (Fig. 6, J) more elongate than that of first stage and endopodite still bears three setae. A spine on disto-lateral part of protopodite in addition to toothed one of first stage.

Now six setae on exopodite of both first and second maxillipeds. Third maxilliped (Fig. 6, L) with functional exopodite bearing six setae and a small bare endopodite which arises at proximal part of basipodite and reaches to its tip. Functional exopodites now on first, second and third pereiopods; those of the first two bearing six setae and that of the third, five. These appendages are similar to third maxilliped except that their endopodites larger; decreasing in size posteriorly. Fourth and fifth legs small and non-functional, but showing indications of segmentation. Pleopods quite large but show no subdivisions.

Third Stage (Fig. 5, D; Fig. 6, M-O). Third stage (Fig. 5, D) about 5.4 mm. in length and similar in color to the earlier stages. Abdomen now composed of six segments and telson (Fig. 6, M), which is more spade-shaped and bears three teeth on each side (with a fine hair between the two lateral), and four pairs of plumose setae medially. There is a median tooth.

Antennule (Fig. 6, N) like that of second stage but plumose setae on margin of peduncle have greatly increased in size and number. Now a swelling proximally. Antenna (Fig. 6, O) large, with endopodite equal in length to exopodite and no longer bearing terminal setae. Segmentation of flagellum of post-larva can be seen through the thin integument of endopodite for some time before ecdysis.

Palp on mandible is now quite large but rest of appendage not appreciably increased in size. Maxillule similar to that of second stage. Scaphognathite of maxilla still narrow.

Six setae on exopodite of first maxilliped and of third pereiopod and seven on rest. No indication of thumb on first pereiopod. Long and slender

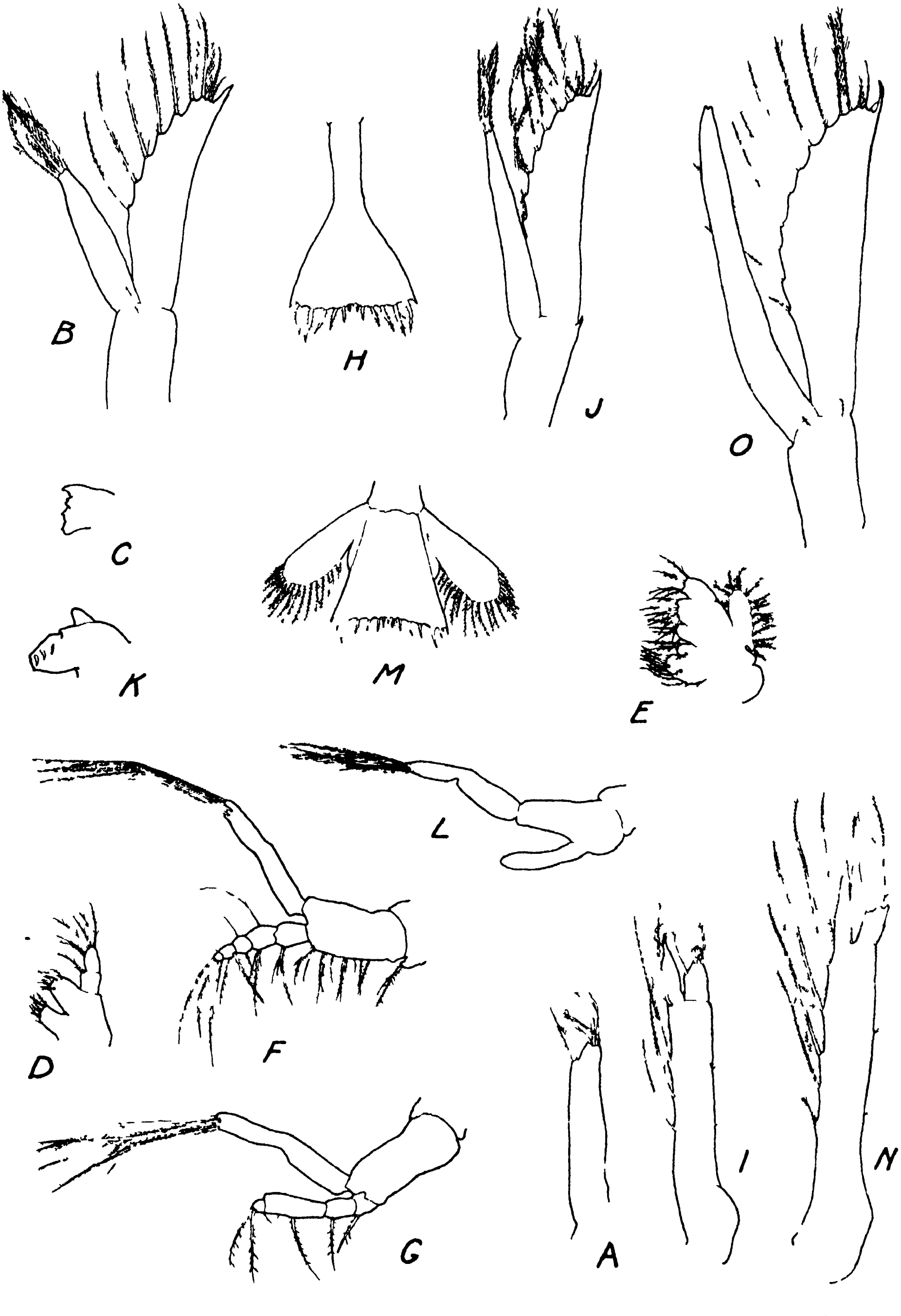


FIG. 6. *Upogebia pugettensis (Dana). First larval stage. A Antennule. B Antenna. C. Mandible. D. Maxillule. E. Maxilla. F First maxilliped. G. Second maxilliped. Second larval stage. H. Telson. I. Antennule. J. Antenna. K. Mandible. L. Third maxilliped. Third larval stage. M. Telson. N. Antennule. O. Antenna.*

anlagen of pleopods show but slight indication of divisions. Uropods separate from telson: exopodite large with about 15 long plumose setae on medial margin but endopodite small and naked.

Post-larval Stage (Fig. 5, E). Post-larva about 4 mm. in length. Very transparent with a few red pigment spots, situated on anterior part of carapace, on first, second, third and sixth abdominal segments, on tips of antennules and on propodi of all the pereiopods. Post-larva (Fig. 5, E) of adult form. Obtuse rostrum with a number of bristles and small knobs distally, and lateral teeth practically obsolete. Abdomen clearly segmented but pleura narrow. Telson rectangular with long plumose setae on distal margin.

Appendages differ from those of adult mainly in clarity of segmentation and in numbers of setae. Flagella of antennule short, inner one of a single segment and outer of about three. Scale of antenna small and square. Mouth parts show no striking differences from those of adult.

A small tooth or thumb on medial distal corner of propodus of first and fifth pairs of pereiopods. Pleopods present on second to fifth segments of abdomen and of much the same proportions as adult appendages. Setae are, however, fewer in number and comparatively longer. Uropods proportionately longer and more rounded than those of adult.

GENUS *Paguristes*

Issel (22) described the larval stages of *Paguristes oculatus* Fabr. from Naples. He gives but two zoeal stages and one glaucothoe, which is a more abbreviated larval history than that of any other hermit crab yet described. It seems possible that he might have missed the normal first stage, as his description of what he considered to be the first stage has the usual second stage characteristics, with the exception of the four setae on the exopodites of the first and second maxillipeds and the non-functional third maxilliped (which, however, in his figure shows short terminal setae).

P. turgidus (Stimpson) has a short larval phase in the laboratory, reaching the post-larval in about one-sixth the average time required for other Anomura. The larvae are large when hatched and increase very little, if at all, in size during development: the post-larva is smaller than the first zoea. The integument is thin and the cast-off skins are easily missed in the culture jars.

As the time spent in each stage is so short, it seems quite probable that Issel might have missed a stage, or confused two. Dr. A. B. Needler pointed out to the writer that all first stage decapod larvae have sessile eyes, which become stalked in the second stage. Issel states quite definitely that they are stalked in his first stage larva (page 348) "i suoi occhi, relativamente piccoli, sono portati da brevissimi peduncoli." His figures do not agree in several instances with his description, *i.e.*, he puts segments in such structures as the third maxilliped so as to make an abnormal (as far as other Anomuran larvae are concerned) appendage. In several appendages he fails to separate the dactylus from the propodus, etc. The unarmed mouth appendages are unusual but might be found in such a species that apparently does not feed in the larval phase. He figures the abdomen of the first stage as composed of seven segments and the telson, which is ridiculous, and especially so as the second stage shows five segments.

The antennule in Issel's figure of the first zoea shows the same degree of development as is found in the second zoea of *P. turgidus* and other Anomura. Also the mandible bears a palp, which is not present in the first stage of *P. turgidus* but develops in the second. The scaphognathite of the maxilla of *P. oculatus* is divided into parts in the same way as that of *P. turgidus.* The pleopods and uropods of the former are in the same state of development as those of the second stage of *P. turgidus.* The metazoea and glaucothoe of both species differ mainly in such characters as might be specific or due to inaccurate observation.

Menon (28) describes a series of larvae from Madras plankton belonging apparently to the subfamily Upogebiinae. These larvae show some interesting intergradations between *Upogebia* and *Paguristes.* The Madras form differs from both of these in having a more prolonged larval life history (five stages, instead of three or four), in the presence of epipodites on thoracic appendages 2-6, and *appendices interna* on the pleopods of the post-larval stage.

The Madras larvae resemble *Paguristes* and differ from *Upogebia* in the presence of lateral spines on the posterior margin of the fifth abdominal segment, and in the absence of a median spine on the telson until the metazoea. These two characters are the main differences between *Paguristes* and *Upogebia* in the early stages.

Paguristes turgidus (*Stimpson*) (Figs. 7, 8 and 9)

Females with freshly laid eggs (which hatched in late August and early September) were found in July near Nanaimo. To judge from the state of the eggs on specimens obtained from the west coast of Vancouver island, the spawning season there is the same as that of the east coast. The egg mass was coral red, changing to yellow as development proceeded.

There are three larval stages and probably only one post-larval. The glaucothoe did not survive long enough to moult again. The glaucothoe was obtained in the laboratory five and a half days after hatching in one instance and eight in another. *Pagurus* usually requires at least 30 days to reach the same state of development, but apparently *Paguristes* takes very little food during development and increases little in size, while *Pagurus* nearly doubles.

First stage about 4.5 mm. in length. Very transparent, with russet-red and yellow chromatophores. Chitin of antenna faintly tinged with blue, and liver yellow. Red pigment in a band anterior to liver; on carapace posterior to eyestalks, medio-laterally and marginally above maxillipeds; on abdomen laterally in paired chromatophores at junction of fourth and fifth segments and ventro-medially at bases of lateral spines of fifth segment: on telson medially and across proximal part: on eyestalks, on bases of antennae, ventrally beneath eyestalks, at attachment of maxillae, on each mandible, on distal part of bases of first and second maxillipeds and on median part of endopodite and exopodite, and on third maxilliped. Yellow pigment medio-posteriorly on carapace; at bases of spines on second and third abdominal segments and margin of spatulate part of telson.

First stage (Fig. 7 A, B) stout. Rostrum narrow and less than one-third length of carapace, which is smoothly rounded posteriorly, resembling in this feature Thalassinid larvae rather than Pagurid. A sharp "branchiostegal" spine. Abdomen stout and dorsum of second segment produced posteriorly into a large median spine. Same regions of third, fourth and fifth segments similarly produced, but as smaller spines. Fifth segment has in addition a pair of spines on the postero-lateral margins. Telson roughly triangular and margined with seven setae on each side; the seventh (lateral) pair simple, the sixth very small and plumose, and the remainder large and plumose: fourth the longest.

Antennule (Fig. 8, A) slightly longer than rostrum, slender with one plumose seta near tip and about six aesthetes apically. Antenna (Fig. 8, B) with stout basis, narrow spinous process ventrally, and broad, flat endopodite, ending in a curved spine and bearing 12 long plumose setae on medial margin. Exopodite cylindrical, with three long plumose setae terminally.

Mandibles (Fig. 8, C) have cutting surface composed of a number of fine teeth. A slight knob on dorsal surface indicative of future palp. Maxillule (Fig, 8, D) with six plumose setae on coxopodite, two spines and two setae on basipodite and a three-jointed endopodite with one seta on distal margin of second segment and three on third. Maxilla (Fig. 8, E) with about ten setae on bilobed coxopodite, nine on basipodite, six on endopodite and ten soft plumose hairs on scaphognathite. An indication of the division of the latter into what is apparently a large exopodite and a small epipodite, covered over by a thin membrane.

First maxilliped (Fig. 8, F) composed of broad basis bearing ten bristles, exopodite with four swimming setae, and endopodite of five segments bearing respectively three, three, one, two and five setae ventrally and long fine hairs on dorsal side of second and third segments. Second maxilliped (Fig. 8, G) with three bristles on basis, four on exopodite and two, two, two and five respectively on segments of endopodite, with fine hairs on dorsal part of second segment. Third maxilliped devoid of setae and non-functional in this stage, but its exopodite reaches to end of basis of second maxilliped and endopodite about half length of exopodite.

Remaining thoracic appendages non-functional but large and well developed and hidden by margins of carapace. First and fifth pereiopods show traces of chelate tips and position of future joints can be seen in all. Fifth pereiopods medially placed and hidden under incurved anterior limbs, which are tucked up under thorax. Anlagen of pleopods not yet apparent.

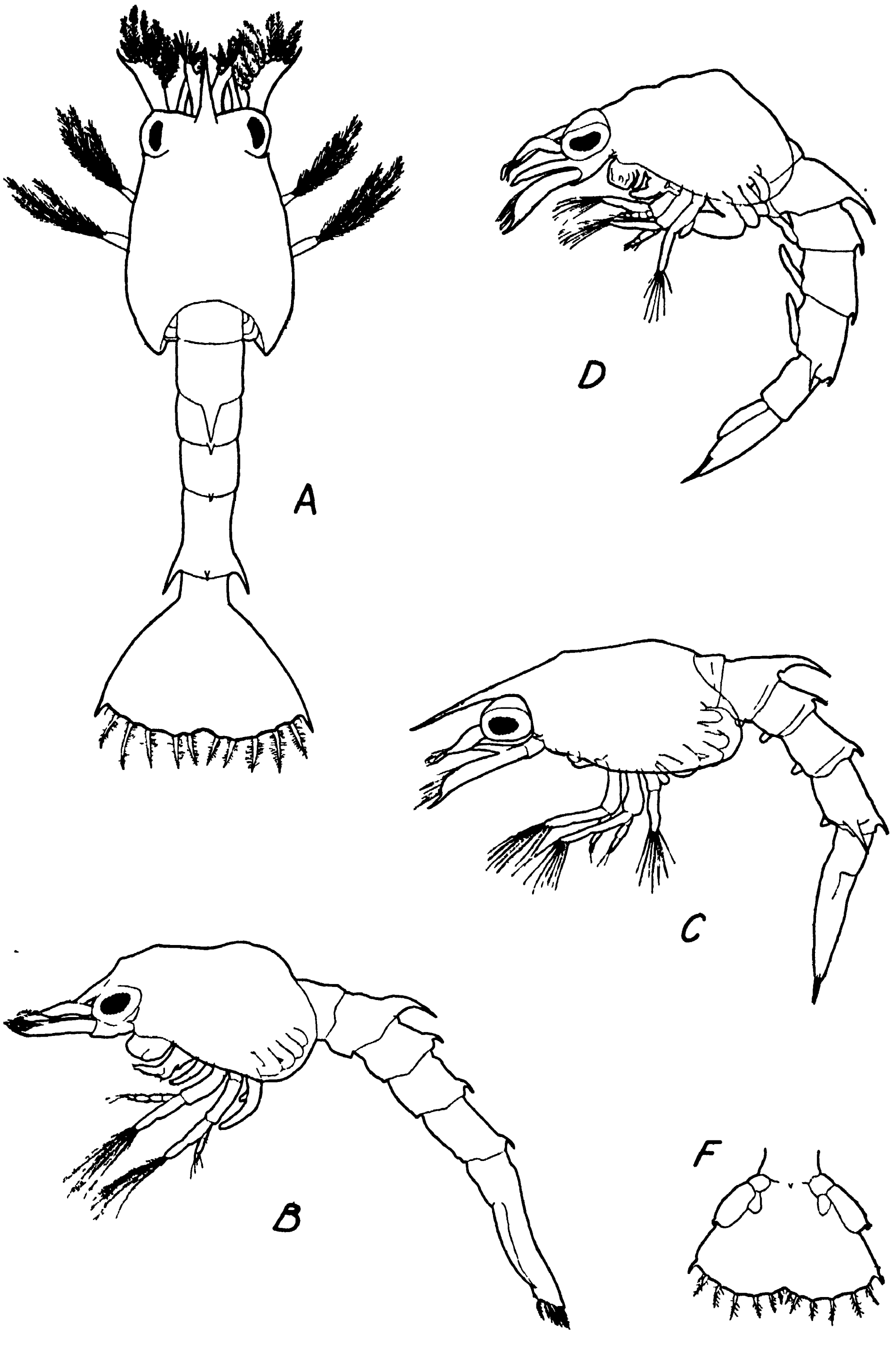


FIG. 7. *Paguristes turgidus (Stimpson). A. First larval stage, dorsal view. B. First larval stage, lateral view. C. Second larval stage, lateral view. D. Third larval stage, lateral view. F. Third larval stage, telson and uropods, ventral view.*

Second Stage (Fig. 7, C; Fig. 8, H-K). Second stage of *Paguristes turgidus* (Fig. 7, C) has not increased in size and its coloration is similar to first stage. Main differences are that eyes now rather elongated and stalked and that sixth segment of abdomen is separated from telson. Telson of same shape but has an extra pair of setae on medial part of posterior margin and the formation of the uropods can be seen through the thin integument. The rudimentary appendages increase in size during this stage so that it would at first glance appear that several stages were present, but there is only one more moult before the post-larval stage.

Antennule (Fig. 8, H) has increased in complexity. Plumose seta now situated on a protuberance, below which are two large plumose bristles, and aesthetes arranged in rows toward tip, which is separate from rest of appendage. Antenna not perceptibly changed.

The unusually well developed mandible (Fig. 8, I) has a naked, knob-like palp. In most pagurid species this does not appear until the last larval or post-larval stage. Maxillule similar except that the two small setae on basipodite have increased in size and are almost as large as the spines, and two additional small setae present now. Maxilla (Fig. 8, J) similar to that of first stage, except that epipoditic part is greatly increased in size and quite bare of setae and separate from exopoditic portion.

Seven setae on the exopodite of first maxilliped; about same number of setae on segments of endopodite but fine hairs of first stage replaced by long fine setae. Second maxilliped has now eight swimming setae on exopodite, and a fine seta on dorsal part of third segment of endopodite. Third maxilliped (Fig. 8, K) has exopodite functional and bearing seven setae. Endopodite quite large, comes off basis more proximally than does exopodite but not yet functional, although showing indications of segmentation.

Pereiopods large and show joints through the thin chitin. Pleopods present as distinct knobs.

Third Stage (Fig. 7, E). Third stage of *Paguristes turgidus* slightly smaller than second and similarly colored. Telson (Fig. 7, E) is of same shape but now two additional pairs of setae on margin medially and uropods separate.

Antennule like that of second stage but segments of appendage of following stage clearly discernible through thin integument. Endopodite of antenna now subequal in length to exopodite and a small spine distally on ventrolateral margin of protopodite.

Palp on mandible considerably larger than that of second stage. Two more small setae on basipodite of maxillule. Epipoditic part of scaphognathite longer and finger-like and bears no setae on margin but exopoditic provided with 16 soft plumose hairs.

Maxillipeds little changed except that first has now eight swimming setae on exopodite. Pereiopods well developed and segmentation of next stage clearly visible. Pleopods large: small endopodite and large exopodite. Uropods with large exopodite, terminating in a curved hook-like spine and with small endopodite.

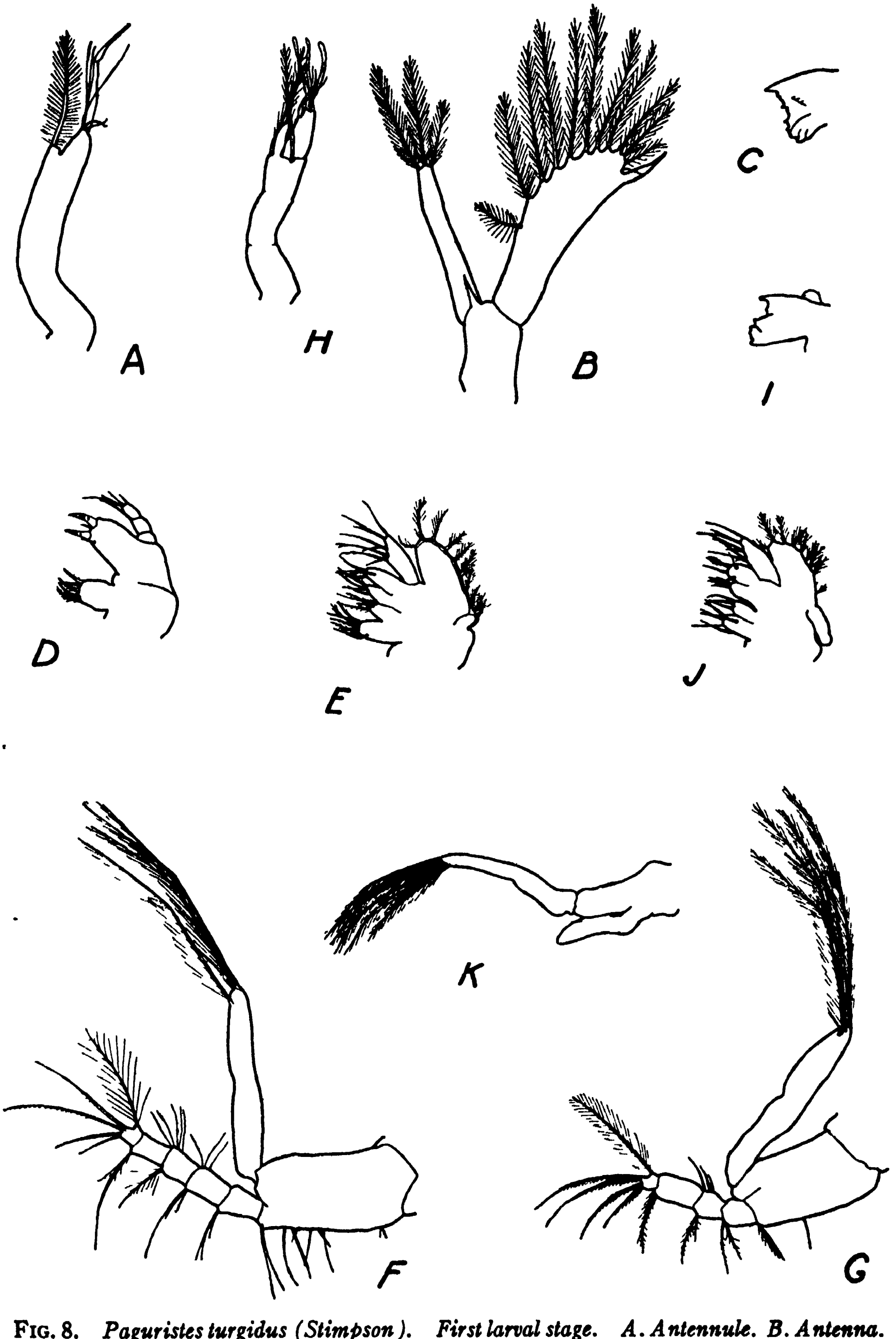


FIG. 8. *Paguristes turgidus (Stimpson). First larval stage. A. Antennule. B. Antenna. C. Mandible. D. Maxillule. E. Maxilla. F. First maxilliped. G. Second maxilliped. Second larval stage. H. Antennule. I. Mandible. J. Maxilla. K. Third maxilliped.*

Glaucothoe (Fig. 9). Glaucothoe (Fig. 9, A) about 3 mm. in total length, with carapace about 1 mm. Of the usual glaucothoe form, but with subequal chelae and only slightly asymmetrical uropods. Like the zoea, very transparent, with yellow tinged legs and russet-red chromotophores scattered over body. Red chromatophores on rostrum; on ventro-lateral edge and median dorso-posterior margin of carapace: a pair on fifth abdominal segment, numerous around margin of telson; on medial dorsal part of eyestalks, on bases of antennae, on distal part of propodi of chelae, on second and third pairs of walking legs, and on each part of uropods. Carapace with blunt rostrum. Segments of abdomen of equal length, but anterior wider than posterior. Telson hexagonal, with two lateral spines and ten long bristles distally.

Antennule (Fig. 9, B) large, with specialized setae and short bristles on first segment of peduncle; inner flagellum two-jointed and small five-jointed outer, armed with scattered bristles and aesthetes. Antenna (Fig. 9, C) has large tooth on peduncle, scimitar-shaped scale and eight-jointed flagellum, with bristles increasing in length and number toward tip.

Mandibles (Fig. 9, D) with well developed palp bearing a few short bristles on distal margin of third segment. Cutting edge rounded in right mandible and somewhat triangular in left. Maxillule (Fig. 9, E) has broad endites with short spines and bristles and endopodite of two segments, of which proximal is large and bears two bristles, and distal very small. Maxilla (Fig. 9, F) has shallow bifid endites with few bristles, finger-like endopodite, and rather narrow scaphognathite, which is divided into exopoditic and epipoditic portions by a slight fold.

First maxilliped (Fig. 9, G) with poorly developed exopodite and endopodite, almost devoid of setae, and narrow endites of protopodite with poorly developed bristles. Second maxilliped (Fig. 9, H) has large exopodite with five plumose setae on terminal segment, and five-jointed endopodite with a few small bristles distally. Third maxilliped (Fig. 9, I) also has large exopodite, with six plumose setae, and five-jointed endopodite with bristles on lateral margin and tip.

First pereiopods are chelate and subequal, with scattered spines and bristles on all segments. Second and third pereiopods long and slender. Fourth pereiopod smaller than second or third, with suctorial setae on propodus, and dactylus short, terminating in a stout claw. Fifth pereiopod also small but chelate: propodus flattened distally and flattened dactylus fits into groove of propodus; suctorial setae and long bristles on tip of appendage. Pleopods all equipped with elongate protopodites, small endopodites each with two ciccinnuli, and exopodites each bearing nine plumose setae. Uropods slightly asymmetrical and each composed of a short protopodite, with a single bristle, a stout exopodite margined with long bristles and suctorial setae, and a short endopodite similarly supplied.

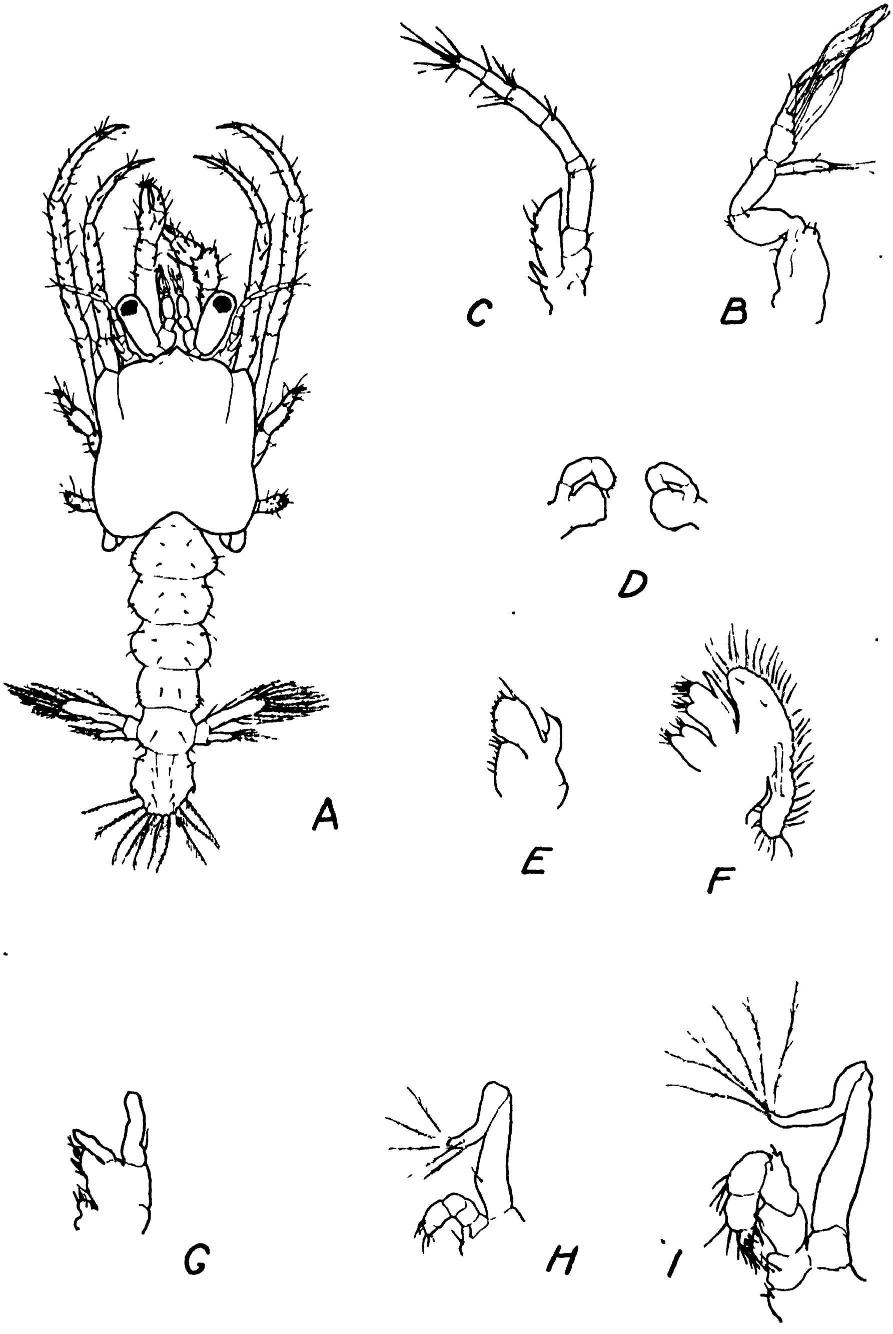


FIG. 9. *Paguristes turgidus (Stimpson). Glaucothoe. A. Dorsal view. B. Antennule. C. Antenna. D. Mandibles. E. Maxillule. F. Maxilla. G. First maxilliped. H. Second maxilliped. I. Third maxilliped.*

GENUS *Pagurus*

Rathke (36, 37) described the larval and post-larval stages of *Pagurus bernhardus*. The only important studies on *Pagurus (Eupagurus)* are by Sars (39) for the European forms and Thompson (45) for New England species. The larvae of *Pagurus beringanus* described below are very like those of *P. bernhardus* as described by Sars, and differ from those of *P. longicarpus* and *P. annulipes* by Thompson mainly in the absence of setae on the endopodite of the antenna in the first two stages.

Pagurus beringanus (Benedict) (Figs. 10 and 11)

Berried females of *Pagurus beringanus* (Benedict) were obtained in March, near Vancouver, and from May until August at different points off Vancouver island. The egg mass changed from brownish black to a grayish brown during the incubation period. The eggs hatched the last day in July and the first young crab was obtained in the laboratory 40 days later; after passing through four zoeal stages and one glaucothoe.

First stage (Fig. 10 A, B) about 2.4 mm. in length. Yellowish in color; rostrum, antennae, mouth parts and maxilliped exopodites blue tinged. Liver pale yellow. Red pigmentation on median anterior part of eyestalks, dorsomedially behind eyes, on carapace above bases of maxillipeds, internally around and beneath anterior part of liver, on bases of mouth parts, on bases of maxillipeds, on dorsal part of third abdominal segment, at junction of fifth segment and telson, and on anterior part of telson.

Body slender and elongate. Carapace has a long sharp-pointed rostrum about equal in length to rest of carapace and over-reaching antennae. Carapace produced posteriorly into a pair of downward curving sharp teeth. Abdomen fairly stout, of five segments, with posterior dorsal margin of second to fifth cut into four teeth, and fifth with long lateral spines in addition. Telson rather wide, triangular-shaped, and bearing seven pairs of spines and setae on posterior, medially indented margin. Lateral pair (seventh) short spines, next (sixth) minute hairs, and remainder, sharp-pointed finely serrate setae, of which the fourth is longest and rest decrease in length, five, three, two, and one respectively.

Antennule (Fig. 10, C) cylindrical, with a long plumose seta subterminally and two aesthetes at tip. Antenna (Fig. 10, D) has a short spinous process on protopodite, a narrow smooth endopodite about three-fourths length of wide exopodite, which has five plumose setae on middle third of medial margin.

Mandibles (Fig. 10, E) of usual type, with fine teeth on cutting edge. Maxillule (Fig. 10, F) has five long and two short barbed bristles on coxopodite, two toothed spines and two setae on basipodite, and no, one and three bristles on segments of endopodite. Maxilla (Fig. 10, G) lamellate, with about nine setae on both bilobed coxopodite and basipodite, seven on endopodite and five soft plumose hairs on narrow scaphognathite.

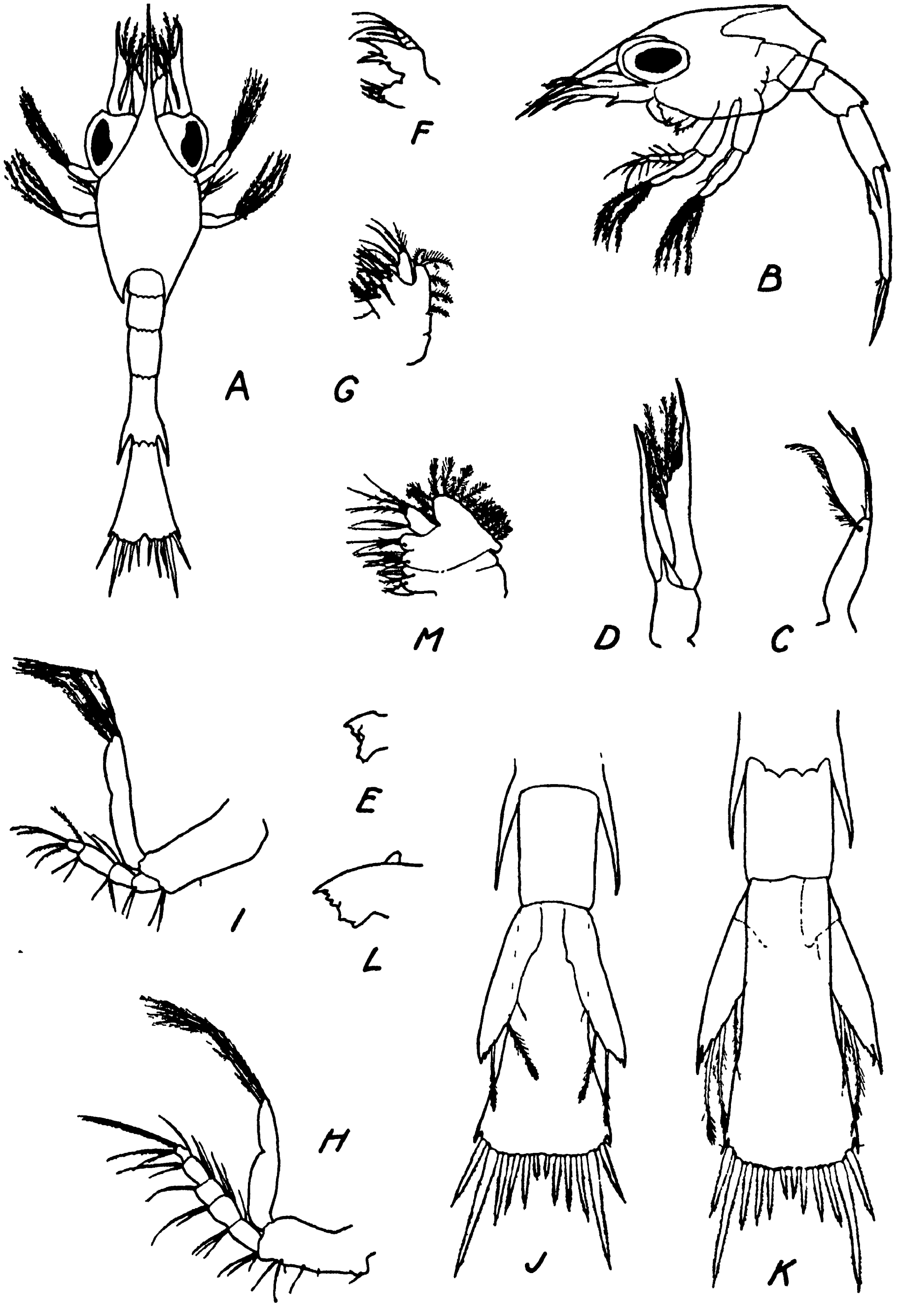


FIG. 10. *Pagurus beringanus (Benedict). First larval stage. A. Dorsal view. B. Lateral view. C. Antennule. D. Antenna. E. Mandible. F. Maxillule. G. Maxilla. H. First maxilliped. I. Second maxilliped. Third larval stage. J. Telson and uropods. Fourth larval stage. K. Telson and uropods. L. Mandible. M. Maxilla.*

First maxilliped (Fig. 10, H) has about nine bristles on basis, four swimming setae on exopodite and three, two, one and five respectively on ventral side of segments of endopodite and soft filaments on dorsal side of first, second and third. Second maxilliped (Fig. 10, I) has three bristles (one serrate) on basis, four on exopodite, and two, two, two and five on segments of endopodite ventrally and filaments dorsally on second and third segments. Third maxilliped non-functional: uniramous and tucked forward under thorax, between bases of other two.

Limb buds small and no signs of pleopods.

Second stage about 2.9 mm. in length and similar in color to first stage. Slight indication of separation of sixth segment of abdomen from telson, but not yet a definite somite. Telson has now an additional pair of small setae medially on posterior margin. Eyes stalked.

Antennule with plumose hair elevated on a small peduncle. Endopodite of antenna now nearly equal in length to exopodite.

Mandibles unchanged in shape. Maxillule has two more spines on basipodite. More setae (eight) on scaphognathite of maxilla than in first stage.

First maxilliped has seven plumose setae on exopodite and filaments replaced by soft plumose hairs on first, second and third segments of endopodite. Second maxilliped also has seven setae on tip of exopodite and a soft hair dorsally on second segment of endopodite. Third maxilliped has six setae on exopodite and no endopodite.

Rudimentary pereiopods slightly larger than those of first stage and appear in lateral view as four knobs covered by carapace. Pleopods not yet visible. Anlagen of uropods can be seen through thin integument of telson.

Third stage (Fig. 10, J) about 3.2 mm. in length. Now six segments in abdomen. Teeth on posterior margins of second and third segments obtuse and lateral spines of fifth segment equal in length to sixth segment. Telson (Fig. 10, J) similar except that uropods now free and fourth pair of setae almost twice as long as any of the others.

Knob on antennule increased in size and aesthetes increased in number. Antenna reaches nearly to tip of rostrum and endopodite longer and wider than exopodite. Small spine on protopodite near base of exopodite.

Mandibles and both pairs of maxillae show no appreciable change except in size.

First maxilliped has seven swimming setae on exopodite and second eight. Third maxilliped has seven and endopodite arises near base of protopodite and is small, with one terminal seta.

Pereiopods still small but tip of first one notched, indicative of chela. Fifth pair hidden under thorax and cannot be seen in a lateral view. Thickenings under integument where pleopods will appear in later stages. Uropods (Fig. 10, J) large, with tip cut into three small teeth, and three stout setae on medial margin; endopodite only a slight protuberance on medial proximal margin.

Fourth stage (Fig. 10, K-M) about 3.6 mm. in length. Differs little from third stage except in size and development of non-functioning appendages, which reach their maximum size in this stage. Sixth segment of abdomen and telson (Fig. 10, K) have elongated considerably.

Two flagella of antennule clearly defined; inner one, bare knob, with a long plumose seta at base and outer with three rows of aesthetes. Antenna similar to that of third stage.

Left mandible (Fig. 10, L) has a sharp dorsal tooth and a wide ventral one on molar process, but right composed of a number of fine teeth. Both equipped with a small bare palp. Maxilla (Fig. 10, M) has about a dozen plumose setae on scaphognathite.

Both first and second maxillipeds have eight swimming setae on exopodites. Third maxilliped has seven, and two setae on endopodite.

Chelate nature of first pereiopod now quite apparent. Pleopods long and slender, but endopoditic part not clearly defined. Exopodite of uropods (Fig. 10, L) now separate from remainder of appendage. Endopodite still just a projection of medial distal corner of protopodite.

Glaucothoe and Adolescent Crab (Fig. 11, A, B). Glaucothoe of *Pagurus beringanus* about 1.9 mm. from tip of rostrum to end of telson. Quite transparent, with yellowish tinge and scarlet and black chromatophores: black on either side of liver, and on bases of third maxillipeds, and red irregularly placed on posterior part of carapace, on second, third and sixth abdominal segments, and on propodi of chelipeds.

Glaucothoe (Fig. 11, A) small but fairly stout, with an obtuse rostrum and unarmed carapace. First segment of abdomen narrow, second wide, and rest taper to rather narrow sixth segment. Telson elongate, and subrectangular with eight terminal setae. Eyestalks large and cylindrical and their scales small and sickle-shaped.

Antennule has an elongate peduncle and inner flagellum longer than outer, which has aesthetes only on proximal half. Appendage sparsely armed with bristles. Antenna with triangular scale and flagellum of about 10 segments.

Mandibles poorly developed and small, with narrow molar portion and fair sized palp. Endites of protopodite of maxillule large, but with few and weak bristles and endopodite small and feeble. Endites of maxilla slightly bilobed and fringed with setae; endopodite small and closely applied to basipodite and scaphognathite narrow.

First maxilliped poorly developed: endites rounded and armed with a few weak bristles on margin, endopodite small and undifferentiated, and two-jointed exopodite tipped with fine hairs. Second maxilliped has a five-jointed endopodite, poorly provided with bristles and a large two-segmented exopodite ending in a brush of fine setae. Third maxilliped well developed, with a large endopodite, well provided with bristles distally and a short exopodite terminating in setae. On the whole the mouth parts are poorly developed and indicate a microplankton diet at this time.

First pereiopod chelate and toothed on cutting margins. Right larger than left. Second and third pereiopods long, slender and simple. Fourth short, somewhat subchelate, and propodus provided with a few suctorial setae. Fifth also small with short dactylus and propodus tipped with three long bristles and several suctorial setae.

Pleopods of three parts with wide protopodite, small endopodite bearing two ciccinnuli and exopodite with eight, nine, eight and six plumose setae respectively. Left uropod slightly larger than right and both composed of short protopodite, wide scimitar-shaped exopodite margined with plumose and suctorial setae and small endopodite bearing one or two setae.

Adolescent crab (Fig. 11, B) like adult except for the usual juvenile characteristics.

Orthopagurus schmitti (*Stevens*) (Fig. 11, C, D)

An ovigerous female of *Orthopagurus schmitti* (Stevens) has not yet been obtained, but on April 27, 1933, plankton was taken off Horswell point, containing metazoeae and glaucothoe of a hermit crab. These were kept in rearing jars until the adolescent stages appeared. These clearly showed the characters and coloration of *Orthopagurus*, so that it is with a fair degree of certainty of identification that these stages are compared to those of hermit crabs and sand shrimps reared from the egg.

Last zoeal stage about 7 mm. in length. Blue tinged antennae, mouth parts and pereiopods, except chelae which are yellow. A mass of scarlet pigment around liver.

Last larval stage (Fig. 11, C) of *Orthopagurus* differs from that of *Pagurus beringanus* mainly in size, color and presence of more setae on certain appendages of former. Carapace produced anteriorly into a long sharp-pointed rostrum and posteriorly into rather obtuse downward-pointing teeth. Dorso-posterior margins of third to fifth abdominal segments cut into four sharp teeth, and lateral spines of fifth segment are about half length of sixth segment. Telson (Fig. 11, D) elongate, like that of *Pagurus*, except that fourth pair of spines are only slightly longer than others.

Antennule like that of *Pagurus* except that there are two long plumose setae at base of inner flagellum, instead of one. Endopodite of antenna reaches to tip of rostrum, and is longer than exopodite, which has nine plumose setae on medial margin.

Mandibles, maxillules and maxillae very similar to those of *Pagurus* except in size.

Maxillipeds like those of *Pagurus* except that they all have eight swimming setae on their exopodites, and that there are three setae on endopodites of third maxilliped. Pereiopods and pleopods of usual degree of development for this stage. Large uropods (Fig. 11, D) nearly as long as telson, and tip of exopodite cut into several elongate teeth, while medial margin bears six plumose setae. Endopodite a small unarmed projection of protopodite.

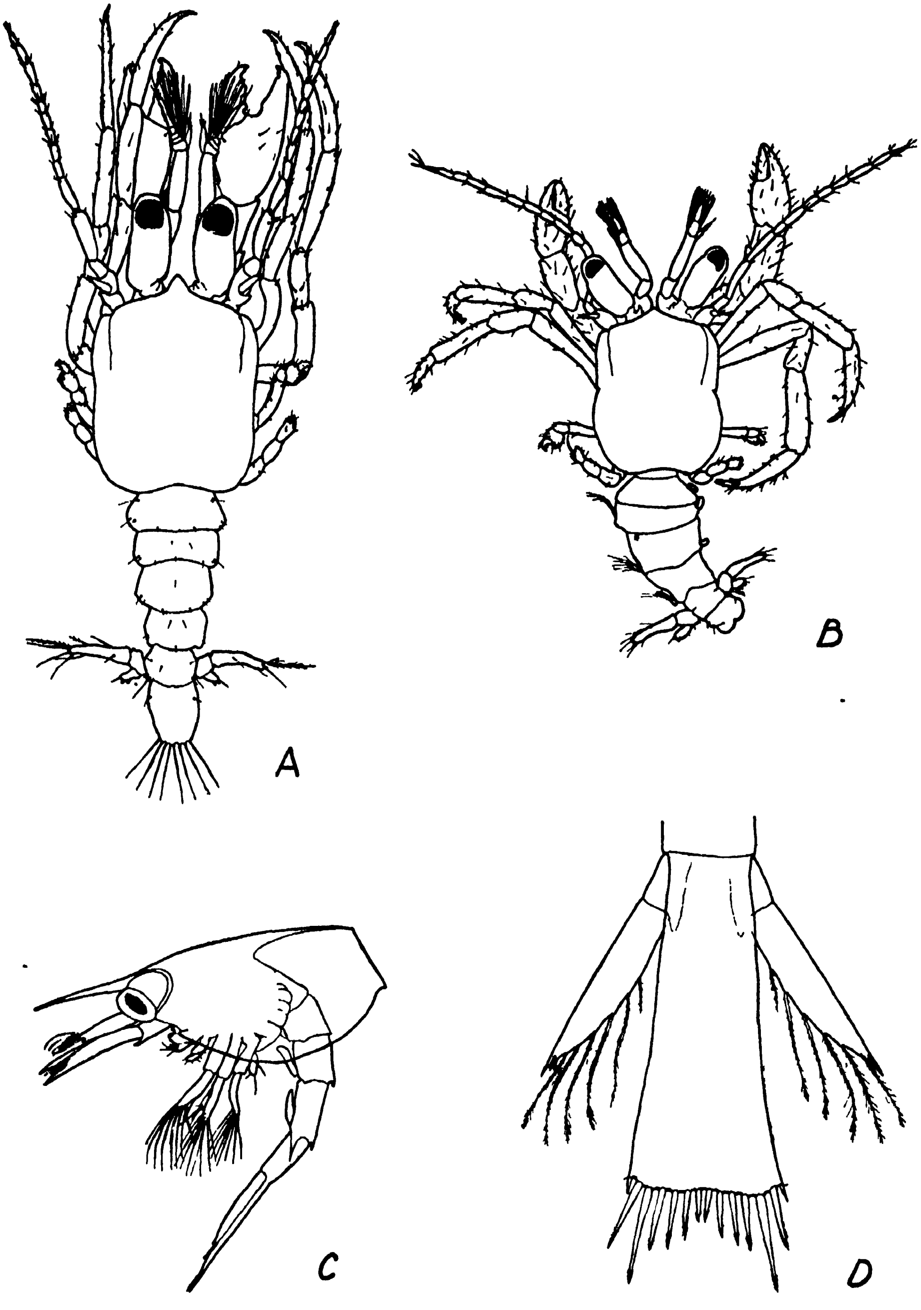


FIG. 11. *Pagurus beringanus (Benedict). A. Glaucothoe, dorsal view. B. First adolescent crab, dorsal view. Orthopagurus schmitti (Stevens). Last larval stage. C. Lateral view. D. Telson and uropods.*

Glaucothoe and Adolescent Crab. Glaucothoe resembles that of *Pagurus beringanus* except that it is nearly twice its size (about 3.5 mm. in length). A pale brown color, with fine red chromatophores on eyestalks and legs, and a large scarlet patch around gastric and heart regions.

The glaucothoe of the two species, in addition to size and coloration, also differ in the form of their chelae and tail-fans. Chelae of post-larva of *Orthopagurus schmitti* are proportionately narrower and fingers longer than those of *Pagurus beringanus*, and inner margins of carpus and propodus are toothed. Uropods longer and narrower and more heavily armed with setae. Telson elongate, with eight long setae on curved distal margin and several short bristles on each of lateral edges.

The adolescent crabs may be distinguished by their subequal uropods and coloration.

Discussion and Conclusions

The present system of classification of Anomura is based entirely on adult characters. As was pointed out by Gurney (16) ontogenetic stages should be of great value in classification and should not be neglected. The adult phase is but one stage in the cycle and therefore should not be given the importance denied another. There naturally are adaptive modifications in the larvae that present difficulties, but such convergences are equally present in the adults, so that this does not necessarily detract from the value of the larvae in a study of relationship. *Upogebia* has but a vestigial antennal scale in the adult phase, while in the larvae, the scale (exopodite), is large and well developed. It is of course essential to realize that the useful characters of the larvae are usually not those structural features used in distinguishing the adults. The conclusions drawn from the small proportion of the larvae that have been studied suggest that the larvae may be grouped by such small differences as the reduction in size of the one pair of setae on the margin of the telson or the absence of setae on the endopodite of the antennae, and these characters would appear to be at least as reliable as many of the adult features that are now considered important in the taxonomy. Unfortunately knowledge of adult morphology is in the majority of cases superficial, and is concerned mainly with the parts that exhibit obvious differences which are of value for ease of separation and of identification. The life histories and larval stages of only a small proportion of the total number of species have been investigated, but larval stages of nearly every family of Decapoda are now known, through the extensive work of Lebour and Gurney particularly. Unfortunately for the problem at hand, the Anomura have received considerably less attention than the other tribes. Hence, morphological information about all stages being scanty and specialization being well marked in the group, generalizations are as yet unsafe; evidence now available permits only tentative suggestions concerning the possible interrelationships.

In the present system of classification the genera *Paguristes* and *Pagurus* are placed in two subfamilies of the family Paguridae. Superficially these are alike, but on morphological grounds, based on both the adult and larval phases, it would appear that their resemblances are the result of convergence due to their specialized mode of life. On the other hand, *Pagurus* and *Orthopagurus* are members of the same subfamily, and this position is confirmed by a study of their anatomy, despite the fact that on the surface they seem more unlike than *Pagurus* and *Paguristes*. *Upogebia*, placed in a different superfamily exhibits a number of characters that are considered primitive. *Paguristes*, in such features as are unmodified by the environment, shows the same primitiveness, which, however, is lacking in the other two under consideration.

In an attempt to determine genetic relationships no hint may be obtained from the study of fossil remains. Fossil Anomura are rare, only the genus *Callianassa* having been found as yet and as early as the Mesozoic era. A few specimens of recent genera, such as *Upogebia*, *Paguristes* and *Pagurus* have been described by Rathbun (35) from Tertiary and Quartenary strata, but obviously these are of no value in this connection. Ortmann (33) believes that the Pagurid group could not have originated before the late Cretaceous or early Eocene Periods. Thus relationships can only be surmised by a study of the living forms, where the problem of distinguishing between environmental and ancestral characters is a difficult one. Thompson (45, p. 197), in discussing the Paguridae, says "In fact, the relationships existing between the various genera as a whole, are very obscure. The group is in all likelihood convergent and not natural, although there is reason to believe that most of the genera are genetically related. If convergent, parts of the group may be of different ages, the characteristic mode of life being assumed at different times by various forms."

One would not expect the mouth parts to be greatly modified by the animal's mode of life, especially where feeding habits are alike, and thus any differences to be seen here should be significant in clarifying our knowledge of their affinities. The maxillules, maxillae and first maxillipeds show such features, *Paguristes* and *Upogebia* having apparently primitive characters which are lacking in *Pagurus* and *Orthopagurus*.

Borradaile (9) gives the characteristics which he considered were possessed by the forebears of the present reptant decapods. Among a number of other features which he believed primitive are a stout, curved, three-jointed palp on the mandible; the basipodite of the maxillule broader than the coxopodite and the endopodite of two segments, the second directed outwards; an epipodite on the first maxilliped; the fourth pair of legs not chelate; and many gills. *Paguristes turgidus* has retained these primitive characters which are lost in both *Pagurus beringanus* and *Orthopagurus schmitti*.

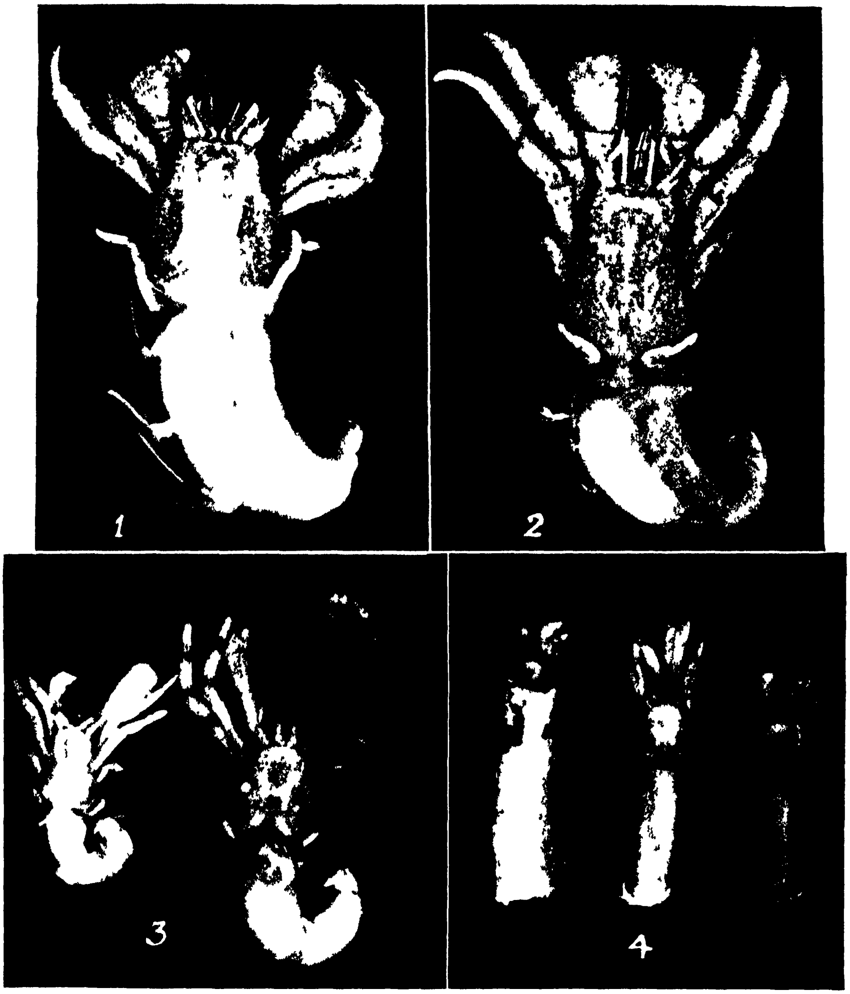
As yet few authors have used the larvae in the classification of decapods. Bouvier (11) considered the larvae in his major divisions. Gurney (16) appreciated their importance and made a number of suggestions for the revision of the systematics, based on larval characters. Lebour (23), from a study of the larval stages of the British Brachyura, pointed out affinities previously unsuspected when the adults alone were considered. She also has made use of her knowledge of the ontogeny of various decapods in the discovery of new species. She found a previously undescribed species of *Caridion* after studying the larvae in the plankton, and in her paper (24) she quotes K. Stephensen—"but it has astonished me that the two larvae are so well distinguished from one another, for the two adult species are so closely allied that I should hardly have dared to consider them different species if their larvae were unknown." (p. 183). Recently (25) she has found another species of shrimp of the genus *Spirontocaris*, and this was also due to the discovery of a different larva in the plankton. Subsequent investigation showed two distinct types of adults among the collections previously considered to be all *S. cranchii.*

It seems probable that the relationships within the group of Anomura should be more clearly discernible among the larvae (which are all free-living components of the plankton and thus comparable) than among the adults, many of which have become highly specialized in correlation with their varied types of environment.

A study of the larvae of the Anomura with which we are concerned here, suggests the same affinities as have been proposed above in the case of the adults. The larvae of *Paguristes* resemble more those of *Upogebia* than *Pagurus*, and the larvae of *Pagurus* and *Orthopagurus* are closely allied. *Paguristes* and *Upogebia* are alike in the number of larval stages, the size on hatching, the thin integument, the stout build, the absence of teeth on the carapace, the short rostrum, and the three plumose setae on the endopodite of the antenna. *Paguristes* larvae resemble those of *Pagurus beringanus* only in the presence of lateral spines on the fifth segment of the abdomen and in the presence of only two barbed spines on the basipodite of the maxillule in the first stage. The last larval stages of *Pagurus* and *Orthopagurus* differ only in size and in number of setae on some of the appendages. One would not expect to find any trace in the larval or post-larval stages indicating that symmetry has been reacquired in the abdomen of *Orthopagurus.* It has been pointed out by Garstang (15) and by Gurney (16), that any recapitulation of such a modification of the ancestral form would be abnormal, as the larvae of the most asymmetrical forms have as typical an abdomen as do those which are bilaterally symmetrical in all stages of their life cycle.

Before a satisfactory system of classification can be obtained, much more study should be directed to the Anomura. Therefore suggestions for rearrangement, with what evidence is at present available, are quite preliminary, and no real changes can be legitimately made until the life histories of a larger proportion of the members of the tribe are known. However, concerning the genera investigated here, it would seem that the evidence justifies the contention that *Upogebia* and *Paguristes* should not be separated by so great a division as is indicated by their position in separate superfamilies while *Pagurus* and *Paguristes* are included in the same family and separated only into different subfamilies. The facts confirm the position of *Orthopagurus*

PLATE I



1 *Paguristes turgidus* (*Stimpson*), *female* 2 *Paguristes turgidus* (*Stimpson*), *male.* 3. *Pagurus beringanus* (*Benedict*), *female and male* 4 *Orthopagurus schmitti* (*Stevens*), *in worm tube, male and female.*

and *Pagurus* within the same subfamily. It is hoped that further study of the life histories of decapod Crustacea will result in a better understanding of the genealogy of the components of the group and in a more natural classification.

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The writer wishes to express her appreciation of the assistance given her by Dr. E. M. Walker in his supervision of the work at the University of Toronto. She is also indebted to Dr. W. A. Clemens for his interest and suggestions during the progress of the experimental work, and would like to thank the members of the staff of the Pacific Biological Station for their helpful co-operation at all times.

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THE MALE GENITALIA IN THE HYMENOPTERA (INSECTA), ESPECIALLY THE FAMILY ICHNEUMONIDAE[1]

I. COMPARATIVE MORPHOLOGY

BY OSWALD PECK[2]

Abstract

The trend of specialization in the male genitalia and the adjacent sclerites is traced within the order Hymenoptera (Insecta) and the morphological problems of these parts discussed. In the Ichneumonidae, these sclerites appear to have little or no tribal or subfamily characteristics of practical value to the taxonomist, such differentiation being masked extensively by specific variation. Thirty-four tribes and ninety-six species were studied. One hundred and fifty-eight figures are shown.

Introduction

In the family Ichneumonidae, the determination of species is often extremely difficult. The keys to the family are based partially upon the characters of the female and cannot always be relied upon for the identification of the males.

Furthermore, it is not at all certain that the present systematic division of the family is a natural one, the situation being summarized by Cushman and Rohwer (32), who expressed the belief that: "The family Ichneumonidae is a group composed of elements showing remarkable differences but at the same time extreme homogeneity. So true is the latter that the grouping into five universally recognized subfamilies leaves the placing of a species in its proper subfamily almost entirely to the imagination or experience of the worker. On the other hand, the strict interpretation of such characters as these keys offer frequently leads even the experienced taxonomist to entirely misplace an insect; and disagreement among workers as to the allegiance of certain genera is very frequent."

This view was supported by Viereck (135) who keyed out the ichneumonid species without defining either the traditional subfamilies or the tribes. The difficulty in defining the higher groups is shown also by Schmiedeknecht (116), who recognized 13 tribes in the Pimplinae, yet keyed out the genera in the family without direct reference to the tribal grouping.

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Joint contribution from the Farnham House Laboratory, Imperial Institute of Entomology, England; the Department of Entomology, McGill University, Montreal, Canada, and the Division of Systematic Entomology, Entomological Branch, Department of Agriculture, Ottawa, Canada. Largely an abstract from a thesis approved for the degree of Ph.D. from McGill University, October, 1936.

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Under these circumstances, it seemed possible that a thorough study of the male genitalia might help to solve these difficulties.

This viewpoint was supported by the presence of tribal characteristics in the ovipositor of the Pimplinae (32), as well as by the constant elongation of the male claspers peculiar to the *Mesochorini*. Furthermore, while specific characters had been shown in the male genitalia of many other hymenopterous groups, this, in itself, did not preclude the presence of tribal ones as well.

In their structural characters, the members of the family Ichneumonidae are regarded as being intermediate between the Chalastogastra (sawflies and horntails) and the aculeate Hymenoptera (ants, bees and wasps). A study of the comparative morphology of the male genitalia in these forms should thus be of value since it may (1) allow us to recognize and define the different stages of morphological specialization existing within the Order, (2) provide data which will assist in making a more rational arrangement of the larger groups within the Order and the families, and (3) aid in the establishment of a consistent, morphologically sound system of nomenclature within the Order.

The term *genitalia* is usually applied to the organs of copulation, which are morphologically external, although it is sometimes taken as including the internal reproductive organs. Studies of the latter are already available in the Ichneumonidae (13–16, 20, 79) and will not be considered here. However, as the specialization of the hymenopterous genitalia in the male usually involves modifications in the remainder of the genital segment, and these, in turn, affect the tenth and eleventh abdominal segments, the study of the genital region was extended to include these segments also, when necessary.

Comparative Morphology of the Male Genitalia

A. THE MALE GENITALIA IN THE HYMENOPTERA

1. *Introduction and Definitions*

An extensive but superficial study of the male genitalia of the Hymenoptera was made by Leon Dufour (34). Supplementary efforts by later workers produced morphological chaos by their failure to give the same names to the same parts. Boulangé (17), by the publication of tables indicating the terms used by the various workers, introduced a semblance of order; however, this author, like Richards (96), preferred to use noncommittal terms, having no precise morphological significance outside of the order, this procedure apparently being based upon the belief, expressed by Richards, that "it is not possible in my opinion to homologise with certainty the parts of the hymenopterous genitalia with those of less specialized orders".

Snodgrass asserted (122, 123) that the parts of the male genitalia in the endopterygote insects can be identified by their musculature and articulatory relationships, although later (124) he described the genitalia "regardless of what may be the morphological relations of the latter". It is certain that in the initial stages of morphological work a non-significant or merely anatomical system of nomenclature is a convenience and, indeed, a necessity; nevertheless, the establishment of a uniform system of nomenclature, based upon morphological homologies, throughout the whole range of the group studied, is, as all agree, the ultimate objective of morphological investigation.

It seems evident that, if homologization is possible between the ichneumonid genitalia and those of other hymenopterous and endopterygote groups, it must be based upon the primitive sub-order Chalastogastra, its parasitoidal family, the Oryssidae, perhaps being intermediate. Boulangé (17), studied the genitalia and their musculature in the Chalastogastra, in *Vespa* and in *Bombus*. He arranged the various structures in series, the members of each group clearly corresponding in position to one another. However, using myology as the criterion, he regarded the inner clasper of *Bombus*, but not of *Vespa*, as homologous with the volsella (or inner clasper) of the Chalastogastra, even though some similarity in form and function existed in *Vespa*.

As will be shown later in this paper, the parts of the male genitalia in the Ichneumonidae are clearly homologous with those in the Chalastogastra and therefore, at least, with those in the Apoidea also.

The male genitalia of the parasitoidal Hymenoptera, so far, have received very little attention, though the genitalia of some chalcids were described by Embleton (36), Imms (56), Grandi (44–46), James (57) and Hanna (51); the development of these appendages in the braconid *Doryctes* was observed by Seurat (118) and their structure in a few species of ichneumonids and braconids was studied by other workers (8, 21, 26, 43, 82, 109).

An effort has therefore been made in the present paper to identify the parts of the male genitalia of the ichneumonids with those of the Chalastogastra and with the more generalized forms of insects. Until this is accomplished, it is obviously impossible to place the terminology upon a sound basis.

As the hymenopterous genitalia have a series of sclerites peculiar to this order, a system of names must be selected for these parts, the choice being according to the claims of priority or custom, preferably the former.

The nomenclatorial systems of the various workers were tabulated by Boulangé (17). The earliest of these were proposed by Audouin (6), Hartig (52), Newport (75), Dufour (34) and Schenck (113). Unfortunately, these workers did not confine themselves to the use of similar terms for parts common to the main groups. The application of the law of priority would result in creating a legal monstrosity derived from several systems and thereby losing most of its value; furthermore Audouin's term *spatha* would have to be applied in a sense that he alone recognized, although the term is widely used for the dorsal portion of the intromittent organ. Under these circumstances practicability is the logical criterion.

The next system in order of priority is that of Thomson (127) and there seems to be little to choose between it and that of Hartig. The latter system was sponsored by Rohwer (101) but otherwise was ignored. The former is followed by Mickel (64, 65), Richards (94-96), Ross (108) and Ries (97) who are some of the chief workers in this field at the present time. Thomson's terms were more widely used than those of any other author and are applicable throughout the order, except, perhaps, with regard to the inner clasper of vespids. They were drawn from the supposed homology or analogy between the gnathal and genital appendages and, to differentiate between them, Crampton (22) suggested that the prefix "gono-" be used for the latter series. To be consistent, it is necessary also to change the term squama to *gonosquama* to prevent confusion with the dipterous squama.

However, Thomson's terms were originally applied to bombids, in which the outer clasper is divided transversely into gonostipes and gonosquama. In the ichneumonids, these are usually fused into a compound structure for which there is no entirely satisfactory term, as the terms gonopod, coxopodite, stylus, harpe and harpago are all morphologically incorrect, while the term stipes-squama of Richards (96) is clumsy, particularly, if the prefix gono- is added. The term *forceps* was chosen in 1841 by Dufour (34) but the same term was previously applied to the dermapterous cerci by Burmeister (18). As the word then is not applicable to the outer claspers in the Hymenoptera, the writer proposes the term *gonoforceps* for the latter structure when the gonostipes and gonosquama are fused together.

Since the word *sagitta* was first used for the penis valve in *Bombus* (127), and since this structure is homologous with the paramere, the term should be sunk as a synonym. However, recent writers have used the term for various sclerites, especially for the articulatory sclerite at the apex of the inner clasper; Thomson named this part the lacinia so that, for the sake of consistency, we should now term it the *gonolacinia*.

Other morphological terms used in this paper conform to the usage of Thomson (127), Boulangé (17) and Snodgrass (122-124), excepting the terms aedeagus and paramere, which agree with some concepts of Snodgrass (122, 123) but not his latest (124). Many of the terms, however, are explained in the text. The hypandrium, outer claspers, inner claspers and harpago are considered as denoting function rather than morphological entities.

2. *The Genital and Postgenital Terga*

Among all endopterygote insects, including the Hymenoptera, Trichoptera, Mecoptera, Lepidoptera, Diptera and Coleoptera, the male genital segment is invariably the ninth abdominal. Behind this segment lies the gonopore or genital opening, in the intersegmental membrane and between the two appendages known as the *gonopods* (122, pp. 17, 18).

Among the Hymenoptera, it is but seldom that the tergum of the genital segment is not radically modified. This tergum may undergo a sequence of changes, the various steps, in order, being: (i) invagination, (ii) division into a pair of lateral *genital tergites* (Tg. IX, Figs. 16, 17, 21), (iii) fusion of these genital tergites with the tergum of the tenth segment to form a *syntergum* (Fig. 20) or, alternatively, fusion of the genital tergites with the tergites of the tenth segment, forming a pair of *syntergites* (Sntt., Figs. 5, 6, 22), and (iv) a reduction in size and functional importance of the syntergites, accompanied often by the loss of the pygopods (Figs. 18, 19).

When the tenth tergum is fused with the ninth, the former may be distinguished by (i) the intimate attachment of the rectum by muscles to the posterior portion of the syntergum, (ii) the points of attachments of the inter- and intra-segmental muscles in comparison with those of the pregenital segments and (iii) the ventro-lateral proximity of the *pygopods* (commonly termed cerci), when these appendages are present. The genital tergites in most ichneumonids have a distinct antecosta; this is absent in the tenth tergum.

In the Chalastogastra, the most primitive group of Hymenoptera in existence, the ninth tergum is usually divided into two lateral tergites. This is true of most siricids (22), although in *Sirex juvencus* L. these tergites are joined by a narrow, sclerotized bridge (17). It is true also of *Xiphidria mellipes* Say (22) and of some species of *Cephus* (17). The incomplete and complete separations of the tergites are shown in *Cephus cinctus* Nort. and *Pteronidea ribesii* Scop. respectively (Tg. IX, Figs. 15, 16).

In contrast to the great majority of the Chalastogastra, the parasitoidal oryssids have the male genitalia completely invaginated (38). In *Oryssus sayi* Westw., as probably in the other members of this sub-order, this invagination has been accompanied by the ninth and tenth terga being reduced in size and in sclerotization, the ninth tergum being divided also into lateral tergites. In this reduction in size, the Oryssidae are more specialized than either the Chalastogastra or the Ichneumonidae. Rohwer (98) noted that cerci (*i.e.*, pygopods) were absent in the Oryssidae; however, in *O. sayi* they are merely concealed by the invagination.

The ichneumonid genital tergum is always divided medianly and may form (i) a pair of tergites (Tg. IX), as in *Pimpla coelebs* Walsh (Fig. 21); this is rare within the family; (ii) a syntergum (Snt), as in *Exeristes roborator* Fabr. (Fig. 20); or (iii) a pair of syntergites, as in *Megarhyssa lunator* L. (Fig. 6) and *Banchus falcatorius* Fabr. (Fig. 22, Sntt.). Intermediate forms also occur.

The chalcids appear to possess a syntergum and a rudimentary eleventh tergum, the syntergum being in many cases identifiable by the possession of pygopods (44-46, 51, 57). It must however be noted that in the figures of these authors the pygopods show that their "ninth tergum" is a syntergum and that Grandi (44-46) incorrectly identified the gonocardo as the tenth segment. The primitive chalcid, *Brachymeria intermedia* Nees, is primitive in this respect also, for the ninth and tenth terga are separate, although the former is medianly divided (Fig. 17).

In the aculeate Hymenoptera, the ninth and tenth terga appear to be always fused (96) and this fusion is usually accompanied by extensive invagination and the loss of the pygopods. In the Chrysididae, only four to six abdominal terga are visible externally (19). Although both Wheeler (137) and Donisthorpe (33) hold that there are ten distinct abdominal segments in the Formicidae, yet the ant *Lasius niger* L. has the sclerotic area of the syntergites almost entirely reduced, although their identity is shown by the prominent pygopods (Fig. 18, Pyg.). Syntergites (Sntt.) are present also in *Vespa maculata* L. (Fig. 19), *V. germanica* F. (17), *Bombus terrestris* L. (17), *Colletes cunicularius* L. (72), *Andrena wilkella* (Kby.) Ill. (Fig. 14), *Halictus lerouxii* Latr. (Fig. 13) and in *Apis mellifica* L. (120). This agrees with the contention of Richards (96) that, in the aculeate Hymenoptera, the ninth tergum is apparently always reduced to a pair of small syntergites.

While it is evident that the ninth tergum, when fused to the sclerite or sclerites posterior to it, is indubitably in direct contact with the tenth tergum, yet the morphological significance of the latter sclerite is not entirely plain. As noted above, the pygopods are borne upon the tenth segment. According to Boulangé (17, p. 218), they are appendages of the tergum, although Middleton (66) has rightly claimed that in *Pteronidea ribesii* they lie ventrally to this sclerite. However, the series of chalastogastrous and ichneumonid adults examined during this study suggest that the pygopods lie immediately postero-ventrally to the tergum, arising in the intersegmental membrane. Snodgrass (122, 124) holds the view that they are not cerci (*i.e.*, not appendages of the eleventh segment) but may be homologous with the socii of the Lepidoptera. Whatever their morphological significance may be, these appendages serve as admirable landmarks of the postgenital segments.

The eleventh tergum varies considerably, both in the degree of sclerotization and in its relationship to the tenth tergum. Berlese (11) claimed that the tenth and eleventh terga were fused together in *Cimbex americanus* L. and separate in *C. axillaris* Pz. This inter-tergal fusion was believed by Crampton to probably form the chalastogastrous epiproct (22). Boulangé (17) found in *Xeris spectrum* L. (Siricidae) and *Cephus pygmaeus* L. (Cephidae) evidence of fusion through the persistence, in an attenuated state, of the musculature of the two postgenital terga. The same author believed that a similar fusion occurred in *Bombus* and *Vespa*.

However, Snodgrass (122, p. 97; 124, pp. 253, 605) considered that the postgenital dorsal sclerite in the Hymenoptera is that of the tenth alone. This is supported by the existence of a broad membranous area behind the last sclerite in many chalastogastrous and ichneumonid species. In the sawflies *Pteronidea ribesii* and *Dolerus unicolor* Beauvois this sclerite bears strong setae and setal alveoli respectively, the setae being similar to those of the tenth tergum; in these cases, the setal remains surely must be landmarks of a primarily sclerotic area, which can only be the eleventh tergum. This identification is also supported by the invariable occurrence of the ichneumonid pygopods in the membrane immediately behind the last functional tergum, suggesting that the eleventh tergum is invariably either fused to the tenth or else de-sclerotized, at least in the ichneumonids.

The further exploration of this problem is outside the scope of this paper, but the morphological value of the pygopods as landmarks makes some reference to it essential, in order to show the significance of the post-genital terga and therefore of the syntergum and the syntergites.

3. *The Genital and Postgenital Sterna*

In the Hymenoptera the ninth sternum may be specialized by (i) invagination, the ninth sternum lying dorsally to the eighth, (ii) reduction in sclerotization and (iii) fusion with the eighth sternum. The hymenopterous *hypandrium* is usually formed of the ninth sternum alone, but in at least some of the Vespidae, the eighth sternum is fused with the ninth. The composite character of the hypandrium in the latter case is usually indicated by the presence of (i) the median *spiculum*; (ii) the *antecostae* of the two sterna and (iii) the inter-segmental and intra-segmental muscles.

The ninth sternum is well developed in the lower Hymenoptera, such as the Chalastogastra (17), the Idiogastra and the ichneumonids, as well as chalcids (44-46, 51, 57). The aculeate hypandrium is reduced in size and lies dorsally to the eighth (17, 70-72, 110-112). Atwood (5) regarded the eighth and ninth sterna of *Halictus* and *Andrena* as formed by a secondary division of the eighth, the gonocardo being the "base of the ninth ventral segment". In *Halictus lerouxii* and *Andrena wilkella*, a strong muscle (Boulangé's *muscle en sangle*) extends from the anterior part of the syntergite (*i.e.*, from the genital tergite) to the ninth sternum and short inter-sternal muscles exist between the eighth and ninth sterna. In *Andrena wilkella* the acrosternites (Ast.) and antecostae (Ac.) of the two sterna are plain (Fig. 14). Furthermore, in this species, the spiculum (Sp.) has been bent posteriorly through invagination, forming an internal median ridge upon the ninth sternum. In Atwood's figures, the antecosta and spiculum of the ninth sternum are plainly shown as darkly stippled areas. In *Apis mellifica* the ninth sternum is "a well-developed semicircular band, forming the ventral and ventro-lateral parts of the ninth segment. It bears on each side, two conspicuous lobes" (Snodgrass (120)). These lobes are the claspers.

Among the wasps, specialization has been carried further by the fusion of the eighth and ninth sterna (17, 58, 132, 138). The vespid hypandrium has been incorrectly interpreted as the eighth sternum and the gonocardo as the ninth by Balfour-Brown (7). The hypandrium of *Vespa* is composed of two sterna (17), while the two antecostae, spiculum, and segmental muscles are also prominent (Figs. 36, 37).

In contrast with the development and subsequent degeneration of the ninth sternum, the tenth and eleventh are always retrograde structures, usually being indefinitely demarcated and semi-membranous. This is plainly due to their early invagination between the terga and the gonopods.

In some ichneumonids, notably *Pimpla instigator* Fabr. and *Megarhyssa lunator* Fabr., the rigidity of the post-genital sterna is still well preserved. All ichneumonids have the ventral margin of the anus and the adjoining rectum supported by a sclerotic area; the latter is usually divided medianly and varies in shape from hyperbolic to V-shaped and quadrate. In *Ephialtes tuberculatus* Auct., *nec* Fourcr., these sclerites bear strong setae with large alveoli, suggesting strongly that these anal structures are not secondarily developed but are remnants of the eleventh sternum, particularly as they lie ventrally to the membranous area that seems to be the eleventh tergum.

In the chalcid, *Brachymeria intermedia*, the post-genital sterna appear to have fused, forming a long rod that joins the lower lip of the anus to the base of the genitalia (Fig. 38); its fusion is not clear and no modification of this kind has been described in other chalcids by Grandi (44-46), James (57) or Hanna (51).

As the anal sterna are fragile and minute, as well as being only indirectly concerned with genitalia, they have not been included in this study; however, they appear to have acquired their present form early in the evolution of the Hymenoptera and may possess group characteristics of interest.

4. *The Genital Appendages*

Since the basal, annular gonocardo is derived from the gonopods, it is an integral part of the male genitalia in the Hymenoptera, although in recent years it has been misinterpreted as the ninth segment by Grandi (44-46), Balfour-Browne (7), Atwood (5) and Abbott (2).

(a) The Typical Structure. The male genitalia differ considerably in the various families of the Hymenoptera, yet all exhibit the same fundamental structure, as shown by Boulangé (17) in the Chalastogastra (Figs. 9-11). Except in the honey bee, four main parts are distinguishable in the adult, these being (i) the basal annular *gonocardo* (Gc.), which bears latero-posteriorly (ii) a pair of hollow, hemi-ellipsoidal outer claspers or *gonoforcipes* (Gf.), each of which supports by its antero-ventral margin (iii) the inner clasper or *volsella.* The gonoforcipes and volsellae support, by means of muscles and membrane, a median intromittent organ, later shown to be (iv) an *aedeagus* (Aed.).

The gonocardo is attached to the intersegmental membrane lying posteriorly to the ninth segment; it sometimes bears a medio-ventral apophysis, the *gonocondyle* (17). Dorsally to the gonocondyle or gonocondylar area lies the tip of the *gonostipital arm*, an anterior elongation of the *gonostipes* or basal portion of the gonoforceps. The apical part of the gonoforceps is frequently differentiated into a distinct appendage, the gonosquama, which may be articulated. Gonosquamae are present in most Chalastogastra (22), in a few ichneumonids and vespids, in bombids and in other aculeate Hymenoptera; in the ichneumonids they are not articulated.

The gonostipes bears the volsella, which, among the lower families, normally lies in the vertical plane. The volsella often assumes bizarre shapes. It frequently bears an apical articulatory sclerite, designated in this paper the *gonolacinia,* although termed the *pièce en trébuchet* by Boulangé (17), the *middle clasper* by Peacock (80) and the *squama* by Salt (109) and Glover (43). The word squama was originally applied by Thomson (127) to the apical portion of the outer clasper in *Bombus.*

The aedeagus has no close articulation with the remainder of the genital sclerites. In its unspecialized form this organ consists of a pair of lateral *parameres*, sometimes termed the *sagittae*, joined both dorsally and ventrally by membrane which may become secondarily sclerotized. The dorsal portion of this membrane may be distinguished as a heavily sclerotized area and then is termed a *spatha*. Each of its antero-lateral corners forms a lateral apophysis, Boulangé's *ergot*, which forms a fulcrum about which the aedeagus can pivot in the sagittal plane; the ergot is usually strengthened by accessory *spathal rods*. Anteriorly the parameres extend far into the body cavity. The aedeagus may assume remarkable modifications in form and size, as in *Bombus*. It is usually large in the chalcids (34, 36, 44-46, 51, 56, 57), dwarfing the small outer and inner claspers. In the hive bee also the aedeagus is abnormally large but, being eversible, it is mainly membranous (120).

(b) Ontogenetic Development. In all Hymenoptera the histoblasts of the male gonopods are situated in the twelfth larval segment behind the head (*i.e.*, the ninth abdominal segment of the adult). This has been demonstrated in the Chalastogastra (17), Proctotrupidae (35), Ichneumonidae (119, 126), Braconidae (42, 76, 131), in the Vespidae, Bombidae and Apidae (138) and in other groups.

A study of the ontogenetic development in *Sirex* (17), in the braconid *Doryctes* (118) and in *Vespa* and *Bombus* (138) shows that each histoblast develops into a primary papilla that divides longitudinally into the outer and inner claspers. A transverse basal sclerite is separated from the base of each primary papilla, fusing with its homologue from the other gonopod to form the annular gonocardo.

According to Zander (138-140) the parameral papilla in Lepidoptera, Trichoptera and Hymenoptera is formed from the base of each primary papilla, soon to fuse with its fellow and with the ejaculatory duct to form an aedeagus. This mode of the formation of the parameres was generally accepted as true until recently, when Mehta (62) claimed that the parameres in Lepidoptera develop earlier than the lobes of the gonopod and independently of the primary papilla. Moreover, Mehta was able to support his ideas by citing evidence from the Hymenoptera (63) and from the other main orders in the Endopterygota. The significance of this is discussed in the next section.

Among the Hymenoptera the formation of the genitalia is always along somewhat similar lines, but variations occur, both in the number of adult appendages and in the order of their differentiation. Unfortunately, most workers upon this aspect studied the genitalia of the honey bee, in which these structures are aberrant: only two pairs of secondary papillae have been found in this insect so either the parameres are absent (120, 138) or else the outer and inner claspers remain undifferentiated (17, 63).

Of greater interest is the disagreement between the developmental and myological evidence. The chronological development in *Sirex*, *Vespa* and *Bombus* is shown diagramatically (Fig. 1); unfortunately, the description of the braconid *Doryctes* by Seurat (118) is inadequate for this purpose. The differentiation of the parameres (Pr.) occurs later than the separation of the individual outer and inner claspers (O.C. and I.C.), both in *Sirex* (17) and *Vespa* (138), although their volsellar muscles are not homologous (17). Yet the reverse is true of *Bombus*, for the parameres are differentiated before the appearance of the two pairs of claspers (138), although the genital muscles are homologous with those of *Sirex* (17).

The discrepancies between these data suggest that the ontogenetic evidence should be interpreted cautiously but suggest the independence of the parameres from the gonopod and thereby provide support for the contention of Mehta (62) that the claspers but not the parameres are gonopodal in origin.

Before our available ontogenetic data can be of value in studies of the hymenopterous genitalia, they must be adequately verified and supplemented. At present they appear to be of little use in comparing the genitalia of adults, unless supported by other evidence.

(c) The Morphological Significance. According to Snodgrass (122, 123), the typical male gonopod of an adult insect consists fundamentally of a basal *coxopodite* and an apical stylus or *telopodite*, the coxopodite bearing an unsegmented *paramere* on its inner margin. The coxopodite may be free, fused to its fellow, or joined to other parts of the genital segment. The parameres of the higher insects are fused to the terminal portion of the ejaculatory duct, forming the median intromittent organ, while the stylus is represented by the clasper; each of the latter may be divided into a pair of claspers also (122, p. 192). Accessory structures may be present but almost invariably they have no muscles and are therefore distinguishable from the paramere and stylus, which are attached by muscles to the coxopodite.

It must be remembered that the origin and position of appendages are not the sole guides to their homologies, for the direction of articulation of limbs is an unusually stable landmark (122). Since the gonopods are believed to be homodynamous with the thoracic limbs, a comparison of the genital claspers with the primitive thoracic limb may be of value in determining the homologies of the genitalia.

The primitive thoracic limb consists of the basal coxopodite (Cxp.) and the distal telopodite (Tlp.) with muscles extending from the base of the coxopodite to the base of the telopodite, as in Fig. 2 (121, 124). The coxopodite moves in a horizontal plane upon the body, the secondarily differentiated coxa moving in the same plane also, while the telopodite articulates vertically upon the coxopodite (121, 124), as shown in Fig. 4. Since both the primary papillae of the thoracic limbs and the genital histoblasts are formed upon the ventral surface of the body, the initial inherent movement of the undifferentiated coxopodite is sagittal. The subsequent development of the insect causes the thoracic papillae to migrate laterally, while the genital appendages move posteriorly; the coxopodite of the latter therefore secondarily articulates in a vertical plane, while the telopodite moves horizontally (121). The significance of this is shown later.

The term paramere was proposed by Verhoeff (133) for a paired appendage lying laterally at the base of the coleopterous penis. Since this time, this term has been applied loosely, especially when the parameres are united as an aedeagus. The location of the coleopterous parameres upon the penis shows that these structures are phallic, not gonopodal, and with this Snodgrass (124) concurs. Yet the parameres of the endopterygote orders are widely believed to be gonopodal in origin, as stated by Snodgrass (122, 123). Snodgrass' conclusions upon the origin of the parameres may also have to be modified if the above-mentioned ontogenetic findings of Mehta (62), prove to be valid. Substantiated by the data of workers in other orders, Mehta's work indicated the probability that the basic data, as outlined by Snodgrass (122, 123), are partially incorrect, the parameres never being gonopodal. Should this be so, Mehta has clarified considerably the current concepts, not only upon parameres, but also upon the fundamental structure of the male genitalia in the Endopterygota, a group in which a common basic form is to be expected.

Recently Snodgrass (124) has reviewed the male genitalia of pterygote insects and his conclusions are quite at variance with his previous ones (122, 123). The term paramere is accepted in the coleopterous sense but in this work he has adopted a terminology "that can be applied consistently to the major structural elements *regardless of what may be the morphological relations of the latter*" (the italics are mine). The genital structures are divided into two classes, phallic and periphallic. The phallic organs are "immediately concerned with the function of coition; they include the phallus and various accessory or supporting structures associated with the latter. The periphallic organs are movable or immovable lobes or processes that have for the most part a grasping or clasping role in the function of copulation" (p. 586). The phallic organs, including the parameres, are evolved from the conjunctival membrane posterior to the ninth sternum or in the genital chamber anterior to the membrane. The "periphallic structures .... arise peripherally, generally from the annulus of the ninth abdominal segment but also from the other segments often closely associated with the latter in the genital complex" (p. 591). They may include a pair of lateral movable claspers and various immovable lobes or processes arising from the tergum or sternum; the movable claspers or *harpagones*. To me, these concepts seem inadequate for the positive identification of some of the genital appendages.

Applying these concepts to the Hymenoptera, Snodgrass suggests that their genitalia are entirely phallic, the gonocardo and gonostipites being formed from the phallobase, while the median appendage is an aedeagus; the gonosquamae "may be termed parameres since they are at least analogous with the parameres of Coleoptera", even though he states that the apices of the parapenes are "structures of the same nature" as the gonosquamae. How little is realized of the morphology of these hymenopterous structures is shown by these suggestions that the parameres may be either the gonosquamae or parapenes, or, if the word aedeagus is to be construed rigidly, even a part of this median organ also.

Snodgrass' conclusions are not entirely consistent with his assumptions, the claspers in the Hymenoptera being considered as radically different from those of allied orders. The claspers in the Lepidoptera arise from the genital cavity (62, 140), as do those of the Hymenoptera (63, 138); yet the former are termed periphallic and the latter phallic, even though their functions are similar. Furthermore, the derivations of the main genital parts in the Lepidoptera, Trichoptera and Hymenoptera are at least similar enough to permit their homologization by Zander (138-140). Even the muscles at the base of the clasper in *Panorpa germanica* L. suggest the affinity of this structure with that of the Hymenoptera (17); yet the mecopterous appendages are termed periphallic. Because of these data, the hymenopterous clasper seems to be homologous with those of allied orders.

The parameres and other genital appendages have been identified also by Pruthi (85). This author considered the parameres to be derived from the median papillae and distinct from the gonopods, which are in the same general plane as the ninth sternum, lying under the penis and parameres. Thus Pruthi agrees with Mehta (62) that the parameres are attached to the base of the penis (it should be noted that Pruthi has interpreted the penis as the aedeagus).

From the ontogenetic data it is plain that the hymenopterous gonopod is basally fused to its fellow, forming the gonocardo (142). This fusion is indicated in the adult by a medio-ventral or medio-dorsal suture or else a corresponding internal ridge; the ventral fusion is shown in many species of ichneumonids, while the dorsal fusion is plainly shown in *Nototrachys foliator* Fabr. and *Trogus lutorius* Fabr. The gonocardo therefore must represent either the two coxopodites or else their bases.

As the median intromittent organ is derived from the fusion of the ejaculatory duct with paired rudiments, this composite organ is evidently an aedeagus. The Chalastogastra, a primitive group in the order, has a primitive aedeagus also, for it often consists almost entirely of the duct, of the two elongate sagittae and of connective membrane; it seems evident that the parameres are represented by the sagittae, especially since the latter are found throughout the members of the order (except possibly *Apis mellifica*) and form the inflexible lateral parts of the compound organ.

The outer and inner claspers are provided with well developed muscles, so that according to Snodgrass (122) neither can be purely accessory structures. Crampton (23, 24) suggested that the gonosquama is the stylus and Richards (96) supported him by indicating that the gonostipes and gonosquama probably form the apical portion of the coxopodite with the stylus. The latter author suggested also that the volsella is a two-segmented paramere and the median organ an aedeagus; his basic definitions evidently differ from those of Snodgrass (122, 123). Becker (8) concluded that the volsella of the Tenthredinidae and Ichneumonidae represents the trochanter and femur, and the bombid volsella the femur. The gonosquama then presumably would be an epipodite. As Becker appears to have compared the hymenopterous genitalia directly with those of the Apterygota, his failure to study intermediate forms seems to invalidate his conclusions entirely.

A homology between the volsella and the telopodite is suggested by the constant presence of the articulating gonolacinia in the lower families of the Hymenoptera. Moreover, the volsella in the Chalastogastra and Ichneumonidae has a constant, diagonal, internal thickening, the *volsellar strut* (V. Str.) (Figs. 9, 117–127). This suggests the remnant of the arthrodial thickening in a flattened limb joint, the adjacent limbs being joined by Boulangé's muscle Q (Fig. 8). However, this strut appears to be secondary, for its function is to prevent buckling of the volsella during the contraction of Boulangé's muscle Q. Furthermore, there are no condyles in the gonostipitovolsellar joint, although they should be present in an articulatory limb that is derived from a primitive leg.

The lack of homology between the volsella and the telopodite is further shown by the base of the volsella being almost flat in the primitive Hymenoptera, so that, if this homology were correct, the telopodite must have changed basally from the normal tubular form to one that has been either very strongly compressed or else split along the main axis of the limb, spread outwards and flattened.

The first method of evolution is impossible, for there is no sign of fusion between the opposite sides of the limb (except possibly the volsellar strut), the basivolsella being thin and almost diaphanous. Furthermore, the intrinsic muscles of the tubular limb must have been gradually replaced by (or developed into) an efficient and complex extrinsic system. It is absurd to postulate the occurrence of both of these radical changes, each of which is highly improbable.

The second method of formation seems to be equally impossible. The longitudinal fission and unfolding of the basal part of the telopodite, accompanied by extensive internal changes, could only have evolved slowly, yet intermediate forms appear to be absent. Furthermore, this change from the tubular form to that of a plate would weaken the telopodite, probably at the very time that it was being adapted as a clasper, a structure that is essentially strong. This theory can be accepted only if accompanied by further proof, and in the absence of a more logical explanation.

The absence of homology between the telopodite and the volsella is shown by the origin of the latter. This structure is formed from the lateral papilla by an apical or medio-apical fission, the volsellar region being the smaller (Fig. 1). This suggests that the volsella is not the entire telopodite, although possibly an endite.

The fundamental difference between the telopodite and the volsella is shown also by musculature. The base of the paramere is joined to the base of the gonolacinia by Boulangé's muscle N in both the Chalastogastra (Fig. 8) and the Ichneumonidae (Fig. 7). Even if it is conceded that the paramere is gonopodal in origin, one cannot recognize the existence of a primary muscle that extends between a basal appendage of the coxopodite and the apical telopodite. Either this muscle is secondary (and secondary muscles are usually short) or else the volsella is not part of the telopodite.

The morphological relationship between the gonopod and the volsella is emphasized by the detailed adult structure of the latter.

The separation of the volsella and gonostipes is anteriorly incomplete in some siricids, as in a species of *Sirex* (39, Fig. 15) and in both *Sirex juvencus* and *Xeris spectrum* (17, p. 228); the same phenomenon occurs in widely separated species of ichneumonids (Fig. 97), and is discussed later. While this is indicative of a similar morphological relationship between the inner claspers of these groups, yet, coupled with the primitive nature of the family Siricidae, it further suggests that the volsella originated from the gonoforceps.

The view that these two structures are primitively one is supported by the appearance of the upright ichneumonid basivolsella, in which (i) setae and alveoli are present facing the aedeagus, (ii) the muscles lie extrinsically and laterally and (iii) the curvature is towards the aedeagus. Together, these factors strongly suggest the rounded shape and musculature of the mesal portion of the gonopod. The setae are evidently primary, because they are small in comparison with the alveoli; furthermore, the setae and alveoli only occur postero-ventrally, as in *Ichneumon grotei* Cress. (Fig. 121), this being a position favored by the basivolsellar curvature for the survival of setae from the attritional effects of invagination, yet an unsuitable place for the development of tactile organs for use during coition. The curvature of the basivolsella is in itself insignificant, for the shape may possibly be due to the tension exerted by Boulangé's muscle Q (Fig. 8), although this muscle probably is only used during coition. Individually these three factors are not of much weight, yet, when correlated with each other and with the ontogenetic development of the volsella, they indicate that the volsella and gonoforceps in the Ichneumonidae are primitively one structure.

These facts, incidentally, serve also to show the falsity of the idea that the volsella may have originated directly from membrane, instead of from sclerotic structures; the form and musculature of the volsella are far too specialized to be associated with such an extensive change, one that is already so complete that there are no intermediate forms, even in the most primitive of the Hymenoptera.

Since the volsella and gonostipes are secondarily divided from each other, the gonopod consists of either three or four units, these being (i) the lateral half of the gonocardo, (ii) the gonostipes and volsella, (iii) the gonosquama and possibly (iv) the paramere of the aedeagus. Unfortunately, these units do not articulate sufficiently in any one plane to allow recognition of the coxopodite, subcoxa or coxa.

Neither can these parts be identified by their musculature. The longest muscles in the male hymenopterons are Boulangé's muscles I, J, M and N (Figs. 8, 10), all of these being attached to the base of the paramere and to the apex of either the gonostipes or the volsella. Consequently, if the paramere is held to be fundamentally separate from the gonopod, these muscles must be attached to the coxopodite and not to the telopodite, the gonostipes and volsella then being derived from the coxopodite. On the other hand, if the paramere is an appendage attached to the base of the gonopod, the gonostipes and volsella must still be derived from the coxopodite, else muscles are extending from the base of one appendage to the base of another. Crampton (24) has cited Snodgrass as considering this derivation to be the case.

However, this conclusion may seem to be invalidated by the gonocardo being constantly the first and only sclerite to be transversely differentiated from the gonopods. On this account, the two coxopodites may be regarded as being modified as the gonocardo. The morphological importance of the latter was emphasized by Pruthi (85), who suggested that it was probably homologous with the structures to be found at the base of the genitalia in the other main pterygote orders.

Assuming that both the gonopod and the thoracic limb are primitively divided into the coxopodite and telopodite, there would seem to be but one way of reconciling the ontogenetic and myological data. It can be put forward that the primary muscles of the gonopod have degenerated, owing to the extensive structural modifications that the gonopod has undergone. As pointed out by Abbott (2), there is no muscle capable of moving the ichneumonid claspers laterally; the genitalia then must be extended sideways by an increase in the blood pressure in this region. Blood pressure has already been noted as a factor in the functioning of the genitalia of the male honey bee (120), although in this insect there are no functional claspers and the aedeagus is mainly membranous. In the ichneumonids, too, the volsella is rotated from the vertical or resting position to the horizontal or copulatory attitude and, as there are no muscles capable of doing so, again an increase in the blood pressure in the neighborhood must be responsible. Should this modification be generally true of the Hymenoptera, then the arrangement of muscles is not a reliable source from which to draw fundamental, morphological conclusions.

The true nature of the hymenopterous gonopod, then, is not as yet definitely established. The median organ is an aedeagus, while the gonocardo *may* represent the fused coxopodites, the remainder then being derived from the telopodite. However, the stipes and volsella are primitively one. Until further evidence is found, it seems impossible to homologize with certainty the hymenopterous genitalia with the primitive limb.

B. THE ICHNEUMONID MALE GENITALIA

1. *The Structure and Musculature*

The ichneumonid genitalia resemble those of the Chalastogastra, a closely related but less specialized group, in which however the genitalia vary greatly in form. The ichneumonids differ from the Chalastogastra in (i) the frequent formation of a syntergum or of a pair of syntergites, (ii) the almost invariable absence of the parapenes, (iii) the absence of a distinct gonosquama in most species, (iv) the greater refinement or specialization of the volsella and its greater uniformity of shape and (v) the greater degree of sclerotization in the aedeagus. The ichneumonids are nearer to the parasitic sub-order Idiogastra, as represented by *Oryssus sayi*, although this species has a non-articulated gonosquama and its aedeagus is only weakly sclerotized.

The main homologies between the chalastogastrous genitalia and those of the ichneumonids are quite evident, if shape, position and articulation are sufficient criteria. This, however, does not apply to the inner claspers, and to show this relationship it is necessary to describe in detail the structure of the ichneumonid inner clasper. This appendage is assumed to be a volsella and the proof is shown subsequently.

The volsella of *Neotypus americanus* Cush. is typical of this structure as it occurs in the ichneumonids so that both the external (or mesal) and internal views of this organ in the vertical position have been shown (Figs. 119, 120). It should be noted that all drawings of this appendage show a large basal piece shaded by broken lines; these lines do not represent a completely desclerotized plate, but, instead, one that is extremely thin compared with the remainder of the volsella, almost resembling membrane in appearance. This plate is termed the *basivolsella* (Bv.) and is braced by the internal *volsellar strut* (V. Str.) which divides the basivolsella by passing from the antero-ventral margin to the postero-dorsal corner of the basivolsella. The anterior apex of the strut is developed into the *basivolsellar apodeme* (Bv. A), which is curved ventrally, laterally or dorsally. The postero-ventral margin of the basivolsella bears the strongly sclerotized *distivolsella* (Dv.) in which the apical margins are usually incurved and fused, forming a hood. The antero-dorsal margin of the distivolsella is prolonged into the *distivolsellar apodeme* (Dv. A.) to which is attached dorsally the gonolacinia (Gl.), which almost invariably is articulated.

The volsellae in most sawflies bear but little resemblance to the inner claspers of the ichneumonids, except that they have in common two distinct sclerites, the anterior *pièce complémentaire* of Boulangé (17) (which corresponds to the basivolsella and distivolsella) and the dorso-apical *pièce en trébuchet* (which is identified with the gonolacinia).

The position and articulation of the ichneumonid inner clasper is quite similar to that of the Chalastogastra. The same variation in articulation occurs in both, since the siricid volsella moves either feebly or not at all upon the gonostipes, although freely in the tenthredinids (17, p. 228); the former is true of a few species of ichneumonids, although the latter is far more common.

The volsellar strut is a constant feature of the ichneumonid inner clasper and is plainly identical in position with the diagonal nervure of Boulangé (17, pp. 62, 97), as shown in the siricids and in the tenthredinid, *Tenthredella mesomelas* L. (Fig. 117). It occurs also in the tenthredinid, *Dolerus similis*

Nort. (Fig. 118), in which the anterior end is developed into an apophysis, corresponding to the anterior apodeme of the ichneumonids.

The apex of the *pièce complémentaire* in both *T. mesomelas* (Fig. 117) and in *D. similis* (Fig. 118) shows a striking similarity to the ichneumonid distivolsella in having the apical hood and the antero-dorsal apodeme. Furthermore, this apodeme is present in *Oryssus sayi* (Fig. 113*) a member of the intermediate, parasitoidal Idiogastra.

Nevertheless, the diversification of form in the Chalastogastra suggests that there is a possibility that these similarities are due only to convergent evolution and thus are insignificant; this is supported by the view held by Boulangé that the inner clasper of the vespids is not homologous with the volsella of the Chalastogastra. On this account, the muscles of the chalastogastrous and ichneumonid genitalia (and the adjacent sclerites) have been compared.

The pimpline *Megarhyssa lunator* was selected for this work since its great size allowed the use of the binoculars instead of the microtome, the lack of appreciable amounts of melanin permitting the satisfactory transmission of light through the sclerites; the elongation of the abdomen and of the genitalia tends to isolate many of the muscles so that they can easily be recognized, but sometimes it also tends to change their axes. The muscles of *M. lunator* are compared with the morphological type of the Chalastogastra, as established by Boulangé (17), whose system of myological nomenclature is therefore followed (Figs. 7–11). Muscles D, E, F and L are not shown in the illustrations of *M. lunator* (Fig. 7) but their positions are described. A few of the muscles and their possible functions in *M. lunator* have already been briefly described by Abbott (2).

The abdomen of *M. lunator* is both narrow and elongate, the sterna being so strongly invaginated that the ventral margins of the terga touch in places. No muscles appear to join the tergum of one abdominal segment to the sternum of the immediately anterior or posterior segment (Fig. 5); the absence of this type of oblique muscle identifies the lateral parts of the syntergites as the genital tergites (Fig. 6), their fusion to the tenth tergites being incomplete posteriorly. The posterior boundary of the latter segment is indefinite, since the tenthredinid pygopods are borne upon the tenth tergum (124), although the ichneumonid pygopods are invariably separated from the tenth tergum by some membrane, this space being accentuated in *Megarhyssa* so that it resembles the eleventh tergum.

The syntergites are joined to the ninth sternum by a well developed muscle, Boulangé's *muscle en sangle*. The hypandrium is joined to the gonocardo by muscles A, B and C; the first two extend from the spiculum to the gonocondyle and to the antero-lateral margin of the gonocardo respectively, lying closely together, as in *Sirex juvencus*. Muscle C is weak but distinct in *M. lunator*, extending between the latero-posterior portion of the hypandrium

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**This figure supports the tentative conclusions held by Crampton (22) in regard to the volsellar structures in the Oryssidae.*

and the gonocondyle. The ichneumonid gonocondyle resembles that of Orthandria in being either minute or absent; the development of the gonocondyle is associated with the lateral torsion of the genitalia in the Strophandria (22)*.

The gonocardo is joined to the basal margin of each gonostipes by muscles D, E, F and G; D and E cross each other ventrally, while F and G are dorsal. Muscle D is attached medianly to the gonocondyle and E similarly to the gonostipital arm. As *M. lunator* has long gonostipital arms, D and E lie dorsally to the gonocardo, E being short. Muscle F extends laterally from the antero-median portion of the gonocardo and is rudimentary, for this part of the gonocardo in *M. lunator* is membranous. Muscle G joins the antero-lateral margin of the gonocardo to the antero-median margin of the gonostipes, and its direction has been changed from being almost transverse to nearly longitudinal, owing to the sagittal elongation of the gonocardo; G has therefore assumed a part of the function of F.

Five muscles, H–L, join the gonostipes to the aedeagus. Muscle H is narrowly attached to the base of the paramere and broadly to the base of the gonostipital arm; it is well developed in *Megarhyssa;* its function is that of raising the apex of the aedeagus, which is balanced about the ergots by membrane and muscles. Muscle I, connecting the gonostipital arm to the ergot, is strong in *M. lunator* and serves to retract the aedeagus, sharing this function with the slender muscle K, which extends antero-dorsally from between the ergots to the gonostipes. Muscle J causes the exsertion of the aedeagus and connects the base of the paramere to the postero-dorsal part of the gonostipes; J is long and strong in *Megarhyssa* and in the Tenthredinids, although short in *Sirex, Bombus* and *Vespa* (17). The length of J in *M. lunator* may be due to the elongation of the gonostipes but then the same should be true of *Vespa*, unless only the apical part of the vespid gonostipital area has been developed posteriorly. Muscle L extends laterally from the ergot to the gonostipes and is both short and weak in *Megarhyssa.*

In *Megarhyssa* the gonosquama appears to be incompletely fused; muscle T is present, extending from the baso-lateral portion of the gonostipes to the gonosquama; the short gonosquamo-gonostipital muscle U and the intrinsic gonosquamal muscle V were not identified in *Megarhyssa*, perhaps because the gonosquama does not articulate in this species.

The volsella articulates only weakly in the siricids but strongly in the tenthredinids (17) so that the muscles of the ichneumonid volsella should resemble those of the latter group. Muscles O and P unite the volsellae to the gonostipites. Muscle O is absent in the Orthandria and in *M. lunator* but joins the base of the tenthredinid basivolsella to the apico-lateral part of the gonostipes. Muscle P, joining the antero-lateral margin of the gonostipes to the distivolsella, is present in all of these groups; it is well developed in *Megarhyssa* and is attached broadly at the base (this is not shown in Fig. 7).

**In the Strophandria or Tenthredinoidea the male genitalia rotate through 180° on the median axis before eclosion; this does not occur in the Orthandria (17, 22, 108).*

Two muscles, M and N, join the volsella to the aedeagus. The former extends from the apex of the gonolacinia to the extreme base of the paramere and is absent both in the Tenthredinidae and in *M. lunator*, although present in siricids. Muscle N joins the chalastogastrous gonolacinia basally to the base of the paramere. A moderately weak muscle is attached to the base of the gonolacinia in *Megarhyssa* but its basal attachment was not definitely established.

The volsella has three intrinsic muscles, Q, R and S, each attached to the base of the basivolsella and extending respectively to the apex of the distivolsella, the apex of the gonolacinia and the base of the gonolacinia. Muscle Q is present in the siricids and is large in *M. lunator;* in the tenthredinids it appears to be attached to the gonolacinia but Boulangé (17, p. 71) is not definite upon this point. In *M. lunator* the attachment is definitely at the basal apodeme of the distivolsella. Muscle R is absent in *Megarhyssa*, as there are no muscular fibres visible within the gonolacinia, while S seems to be reduced to some weak fibres.

The muscles of *M. lunator* disagree in detail with those of either the Orthandria or Strophandria alone, but when these are considered together, there can be no doubt that the gonocardo, gonostipes, gonosquama, basivolsella, distivolsella, gonolacinia and aedeagus are homologous in the three groups, the basi- and distivolsella together corresponding to Boulangé's *pièce complémentaire.*

2. *Function*

While much has been written upon courtship among the Hymenoptera, including some ichneumonids, only a few inadequate references have been made to the function of the genital parts of the male ichneumonid during coition or, indeed, even to the coital attitude of the male.

As early as 1799, De Geer (41) described the seizure of the female subgenital plate (*i.e.*, the eighth sternum) by the outer claspers of a male ichneumon, while Rohwer (100) noted that the inner claspers and aedeagus in the sawfly *Euura macgillrayi* Roh. were inserted into the genital cavity at the base of the subgenital plate. Neither Boulangé's own work nor his review of the literature adds anything further in regard to the functioning of the inner claspers and aedeagus.

The usual ichneumonid position of copulation seems to be that of *Pimpla instigator*, in which the male is dorsal to the female with his abdomen curved below the tip of the female's and somewhat to one side in order to avoid the ovipositor. In *Megarhyssa lunator* the male lies above the female with the apex of his abdomen below that of the female and the aedeagus is inserted from the anterior direction, since the female gonopore opens anteriorly (1, 2). In *Paniscus*, the male finally assumes a pendent position (130); this may be true of *Angitia fenestralis* Hlmgr. also, for specimens taken *in copula* were only loosely held together after being killed in alcohol and this may be due, not to chemical reactions upon the muscles, but to the adults having died before the pendent attitude was reached. This variation in copulatory position suggests that there may be a correlation between the coital attitude and structure, as suggested by Abbott (2).

In this regard, it is interesting to note that in *Xiphidria* (Chalastogastra : Orthandria) the copulation is dorsal (100), there being no transverse rotation of the appendages in this group (17), although in at least some of the Strophandria, the males and females mate while facing in opposite directions (17, 55, 100).

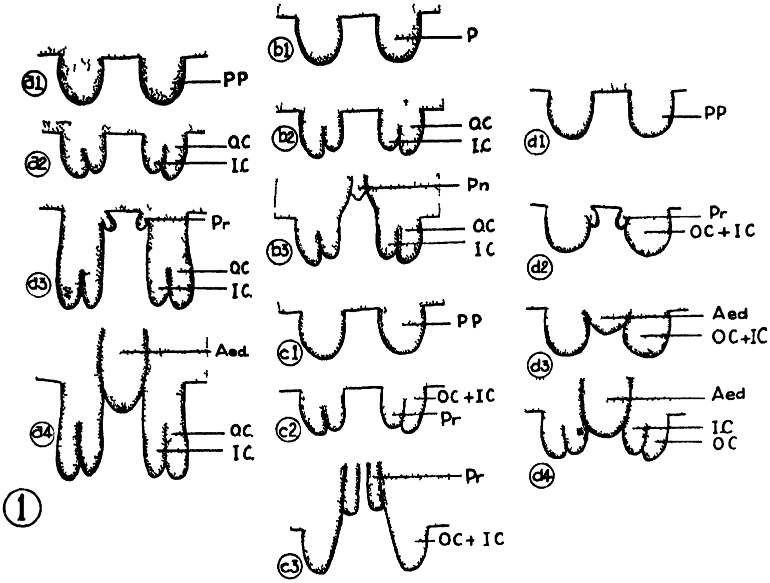
Specimens of the ichneumonid, *Angitia fenestralis*, taken *in copula*, were examined. In this species, the distal parts of the outer claspers grip the lateral portions of the subgenital plate of the female (Fig. 12), who no doubt extrudes her gonopore at the same time. The volsellae are rotated into the horizontal plane, owing to the muscular, ventro-posterior exsertion of the aedeagus and the increase in the blood pressure. The distivolsellae are bent upright through the contraction of muscle Q and the volsellar pincers are directed dorsally in respect to the male (but ventrally and slightly anteriorly with reference to the female). In this position, the pincers of the volsella are closed upon the intersegmental membrane of the female, owing to either the gonolacinial muscles or to the exserted aedeagus pulling the base of the gonolacinia posteriorly. The function of the volsellae is apparently to seize the conjunctival membrane, keeping it taut so that the aedeagus can be inserted into the female gonopore and be retained there. The aedeagus is exserted with its apex directed ventrally and, as it is strongly curved ventrally in this species, both its shape and its position at right angles to the main axis of the genitalia assist to retain the aedeagus in position during copulation. It should be noted that the function of the volsella was not definitely shown by dissections, because of the relaxations of the muscles but these conclusions were strongly indicated.

(To be concluded in next issue.)

ABBREVIATIONS USED IN THE ILLUSTRATIONS

The abdominal segments are numbered with Roman numerals. The muscles of the male genitalia are lettered from A to V in accordance with the system of Boulangé (17) for the Chalastogastra.

Ac.	antecosta	Ov.	ovipositor
Aed.	aedeagus	Ov. S.	ovipositor sheathe
Ast.	acrosternite	Pn.	penis
Bv.	basivolsella	P.P.	primary papilla
Bv. A.	basivolsellar apodeme	Pp.	parapenes
Cx.	coxa	Pr.	paramere
Cxp.	coxopodite	Ptar.	pretarsus
D.A.	dorsal area of the basivolsella	Pyg.	pygopod
D.M.	depressor muscle of telopodite	Scx.	subcoxa
Dv.	distivolsella	Snt.	syntergum
Ejac. D.	ejaculatory duct	Sntt.	syntergite
Erg.	ergot	Sp.	spiculum
Fm.	femur	S. Pl.	sensory plate
Gc.	gonocardo	Spt.	spatha
Gf.	gonoforceps	S-s.	intersternal
Gl.	gonolacinia	St.	sternum
Gsq.	gonosquama	Tar.	tarsus
Gst.	gonostipes	Tb.	tibia
Gst. A.	gonostipital arm	Tg.	tergum
Gtt.	gonotergite	Tlp.	telopodite
I.C.	inner clasper	T-s.	tergo-sternal
L.M.	levator muscle of the telopodite	T-t.	inter-tergal
M.	muscle	V.A.	ventral area of the basivolsella
Mb.	membrane	V.R.	ventral ridge of the aedeagus
O.C.	outer clasper	V. Str.	basivolsellar strut



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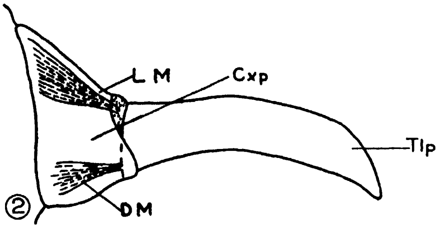

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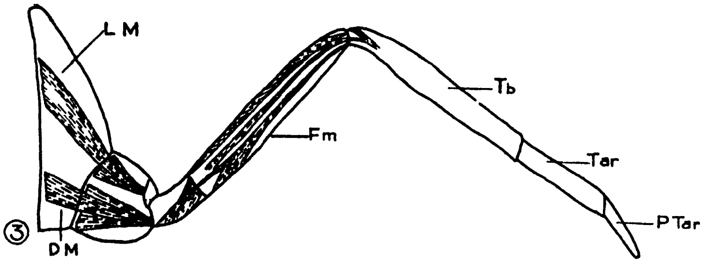

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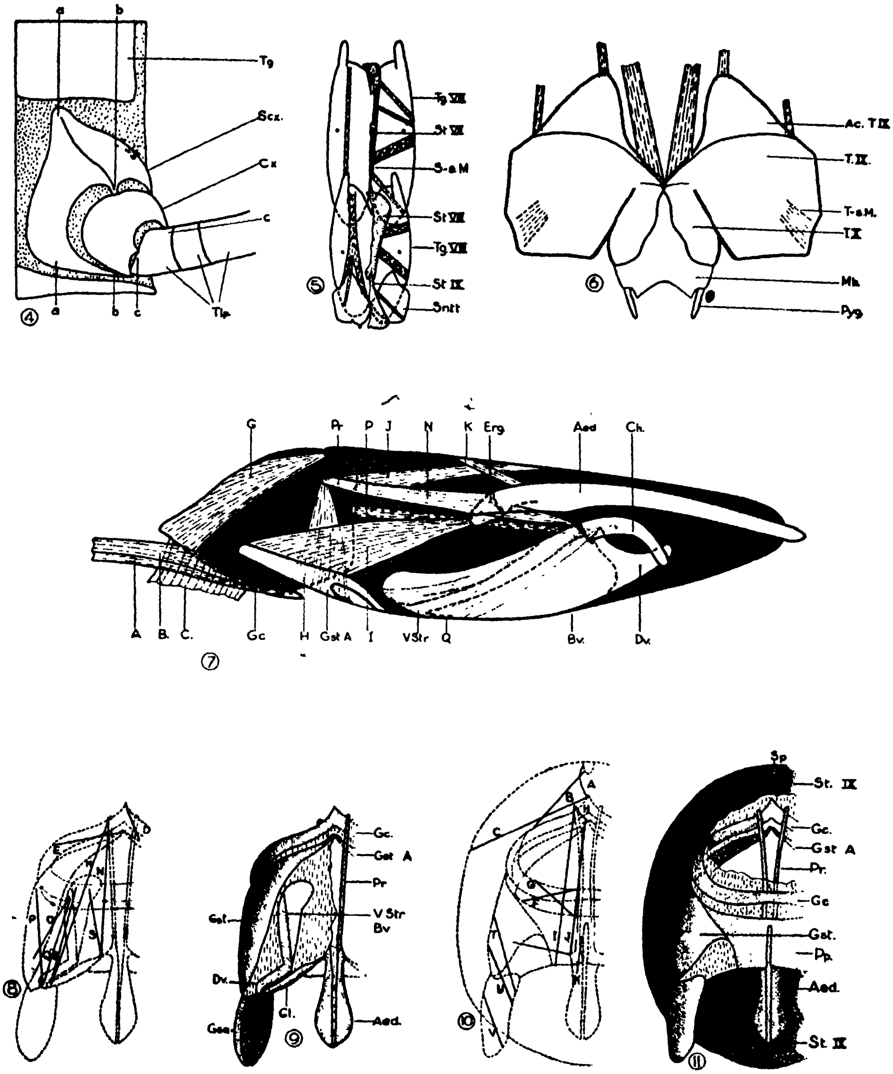
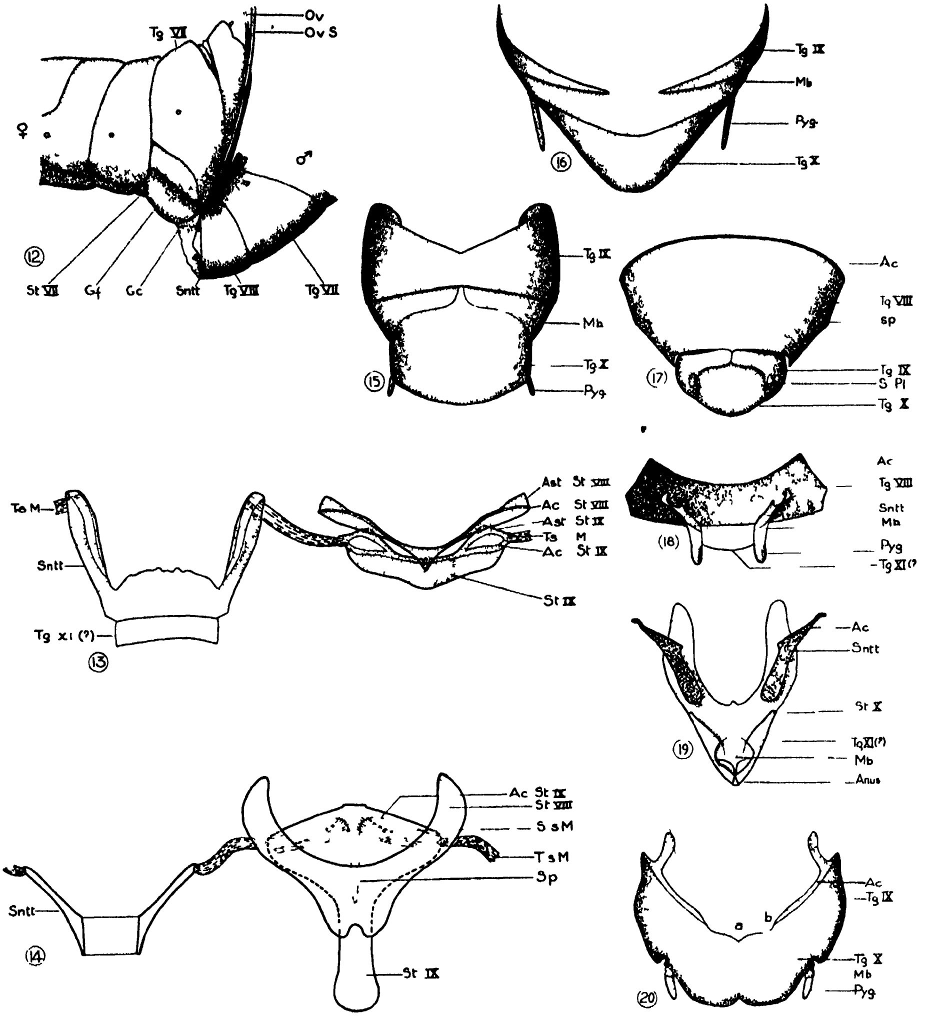
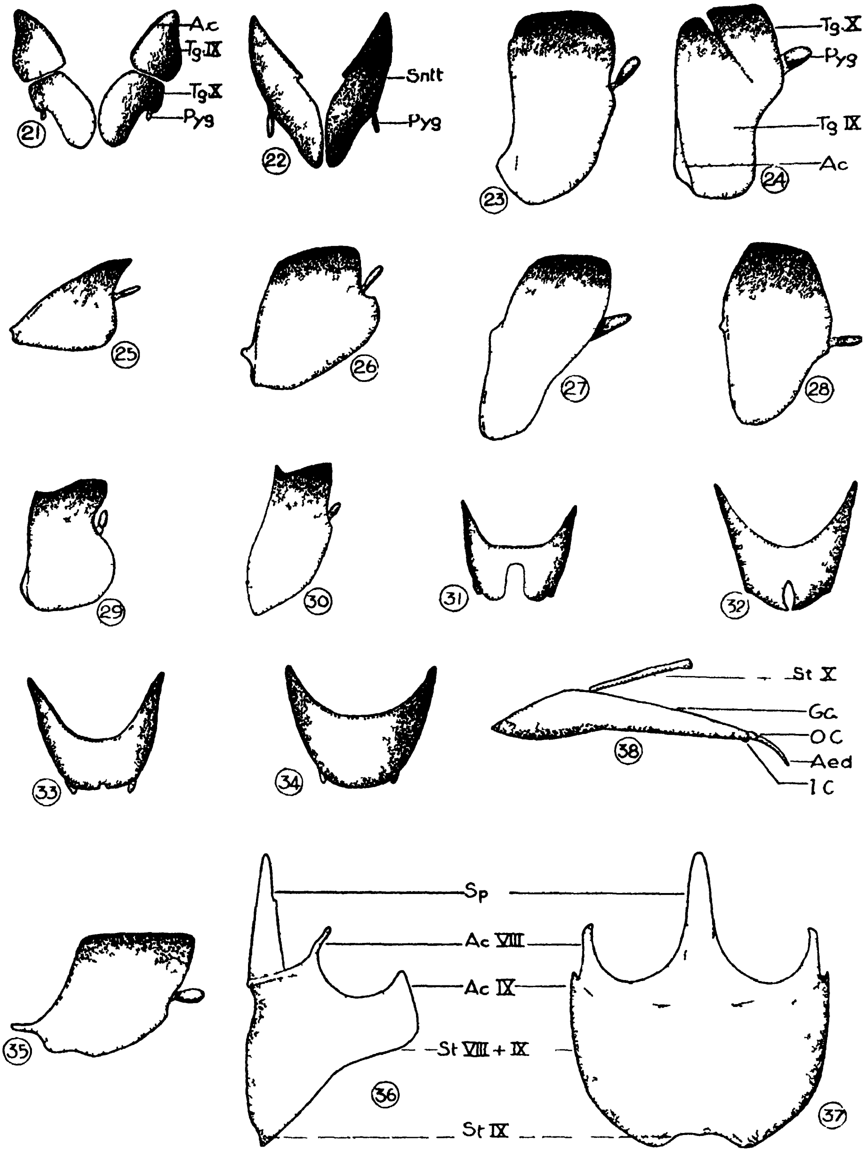
FIGS. 1–3. *Diagrams showing the developmental stages of the male genitalia in: (a) Sirex (according to Boulangé (17) and Vespa (Boulangé, after Zander (138). (b) Apis mellifica (according to Snodgrass (120), after Zander (138). (c) Apis mellifica (according to Michaelis (63) (d) Bombus (according to Boulangé, after Michaelis (63). 2. Diagram of the coxopodite and telopodite of a primitive limb, showing the levator and depressor muscles of the telopodite (after Snodgrass (124). 3. Diagram of a secondarily divided limb, showing the development of the secondary muscles (after Snodgrass (121, 124).*



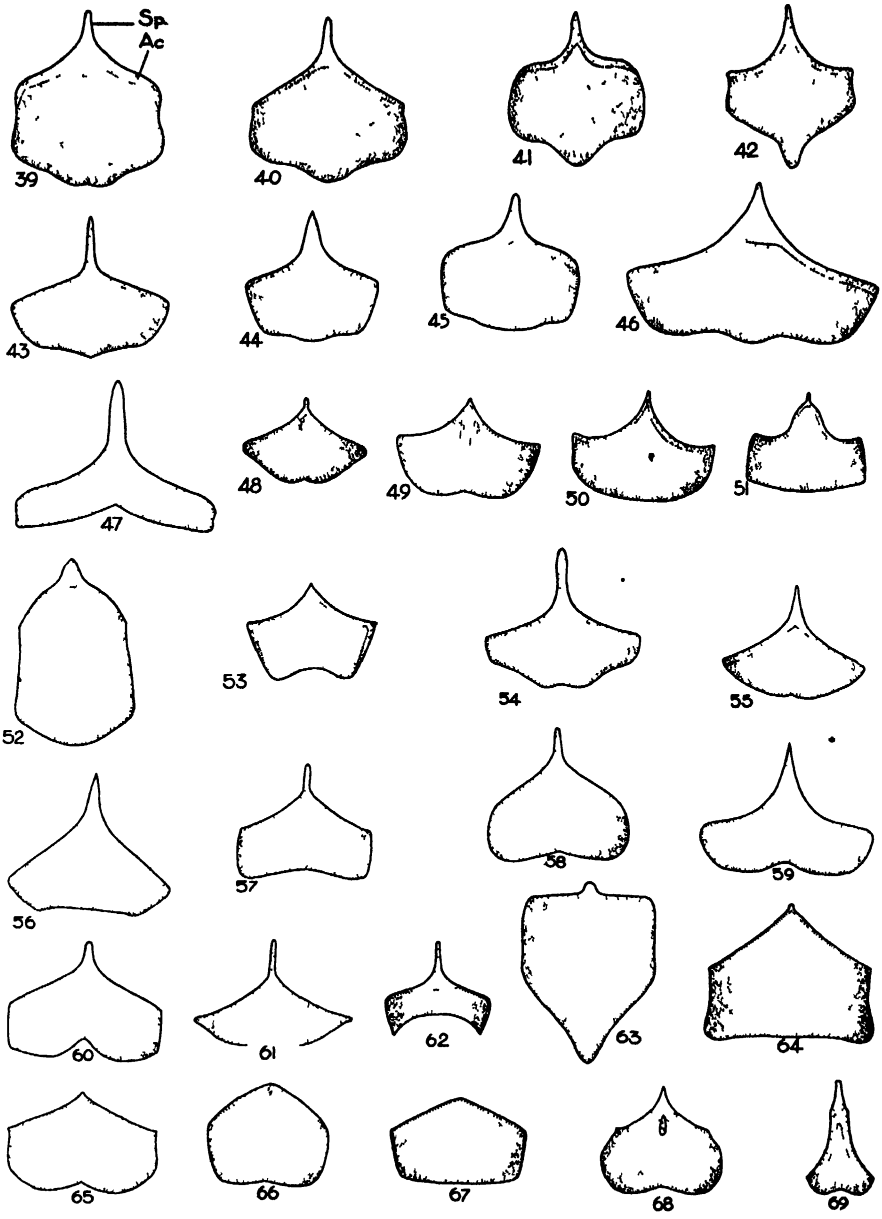
FIGS. 4–11. 4. *Diagram of a thoracic limb, illustrating the subcoxa, coxa and telopodite, together with their basal axes (a-a, b-b and c-c respectively) about which they move (modified after Snodgrass (122).* 5. *Megarhyssa lunator. Ventral view of abdominal segments VII-X, the terga spread horizontally and the left side of each sternum removed; tergal muscles placed to the left of the stippled sterna, the sternal and tergo-sternal muscles to the right.* 6. *Megarhyssa lunator. Dorsal view of the syntergites and their muscles, the syntergites placed in the horizonal plane.* 7. *Megarhyssa lunator. Inner aspect of the right half of the genital appendages, showing the muscles (exclusive of muscles D, E, F and L).* 8–11. *Diagrams of the genitalia and genital muscles in the Chalastogastra.* 8. *Ventral view of the genital muscles, the volsella placed in the horizontal plane; sclerites shown by broken lines and muscles by solid lines (redrawn after Boulangé, 1924).* 9. *Sclerites in Fig. 8 shown in relief; membranous areas shown by broken lines.* 10. *As in Fig. 8, but dorsal.* 11. *As in Fig. 9, but dorsal.*



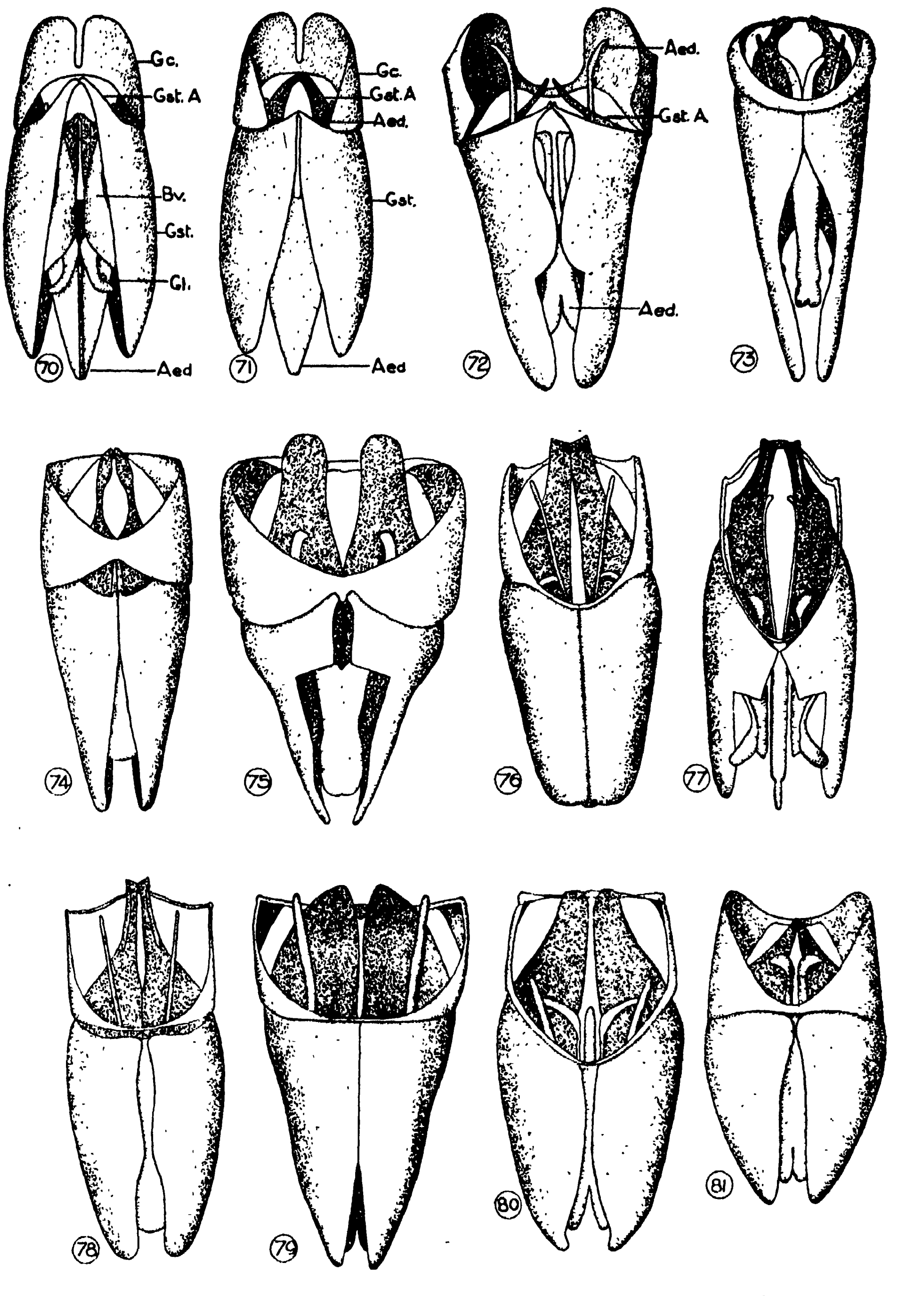
FIGS. 12–20. 12. *Angitia fenestralis. Lateral view of the terminal abdominal segments during copulation.* 13, 14. *External view of the syntergites, the eighth and ninth sterna, and the connecting muscles; stippled to show the degree of sclerotization.* 13. *Halictus lerouxii (Apoidea).* 14. *Andrena wilkella (Apoidea).* 15. *Cephus cinctus (Cephidae). Dorsal view of the terminal abdominal segments.* 16. *Pteronidea ribesii (Tenthredinidae). As above.* 17. *Brachymeria intermedia (Chalcididae). As above.* 18. *Lasius niger (Formicidae). As above.* 19. *Vespa maculata (Vespidae). As above.* 20. *Exeristes roborator (Ichneumonidae) Dorsal view of the syntergum, showing the line of median fission in the tenth tergum (a), and the line of inter-tergal fusion (b)*



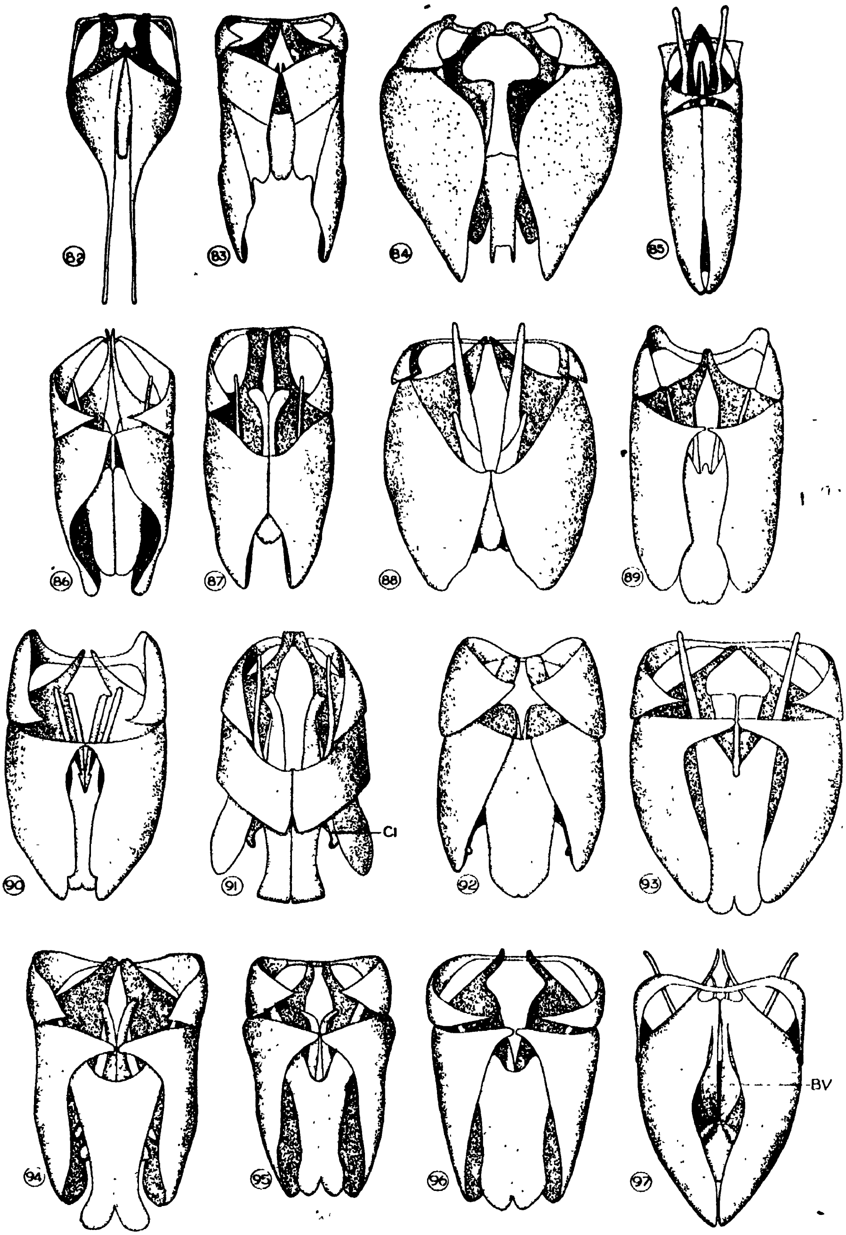
FIGS 21–38. 21–35 *The ninth and tenth terga, the antecosta (Ac) shown by a dotted line.* 21. *Pimpla (Iseropus) coelebs. Dorsal view of the tergites of the ninth and tenth terga.* 22 *Banchus falcatorius. Dorsal view of the syntergites.* 23. *Glypta fumiferanae Lateral view of the ninth and tenth terga* 24 *Glypta rufiscutellaris. As above.* 25. *Theronia fulvescens. As above.* 26. *Theronia melanocephala. As above.* 27. *Exetastes fascipennis As above* 28 *Exetastes matricus. As above* 29. *Exenterus claripennis As above.* 30. *Exenterus marginatorius. As above.* 31. *Cremastus flavo-orbitalis Dorsal view* 32 *Cremastus geminus As above.* 33. *Cremastus minor. As above.* 34. *Cremastus incompletus As above.* 35. *Itoplectis conquisitor Lateral view* 36. *Vespa maculata (Vespidae). Lateral view of hypandrium.* 37. *Vespa maculata (Vespidae). Ventral view of hypandrium.* 38. *Brachymeria intermedia (Chalcididae). Lateral view of the male genitalia and the tenth sternum.*



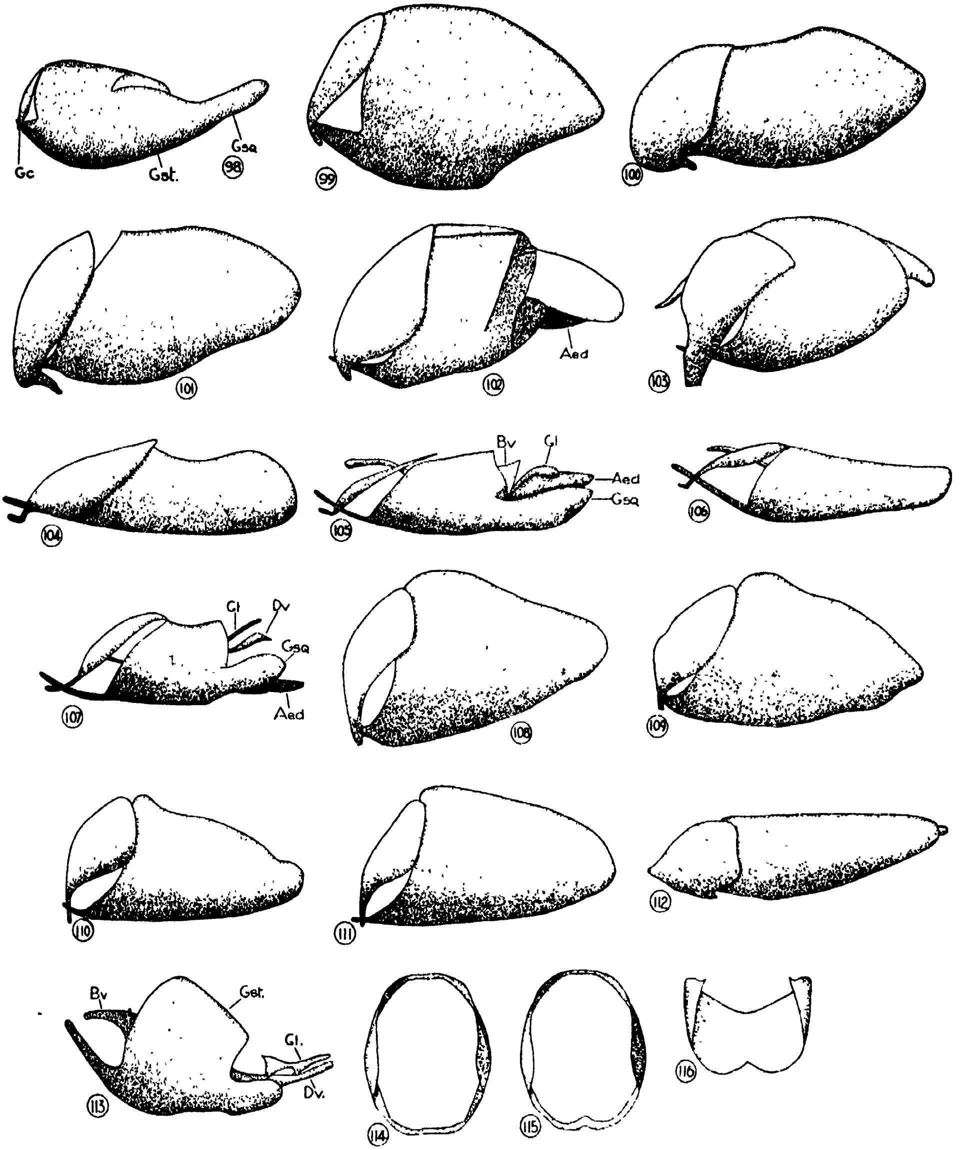
FIGS. 39–69. *Ventral view of the ninth sternum; membranous areas shown by broken lines.* 39. *Ichneumon variegatus.* 40. *Ichneumon animosus.* 41. *Ichneumon perscrutator.* 42. *Ichneumon grotei.* 43. *Phaeogenes gaspesensis.* 44. *Phaeogenes hariolus.* 45. *Hemiteles hemipterus.* 46. *Hemiteles subzonatus.* 47. *Hemiteles fulvipes.* 48. *Exetastes fascipennis.* 49. *Exetastes matricus.* 50. *Glypta rufiscutellaris.* 51. *Glypta fumiferanae.* 52. *Theronia fulvescens.* 53. *Theronia melanocephala.* 54. *Omorgus borealis.* 55. *Omorgus ensator.* 56. *Omorgus mutabilis.* 57. *Cremastus incompletus.* 58. *Cremastus flavo-orbitalis.* 59. *Cremastus minor.* 60. *Cremastus geminus.* 61. *Cremastus interruptor.* 62. *Pimpla coelebs.* 63. *Pimpla instigator.* 64. *Exenterus claripennis.* 65. *Exenterus canadensis.* 66. *Exenterus marginatorius.* 67. *Exenterus lepidus.* 68. *Tryphon incestus.* 69. *Megarhyssa lunator.*



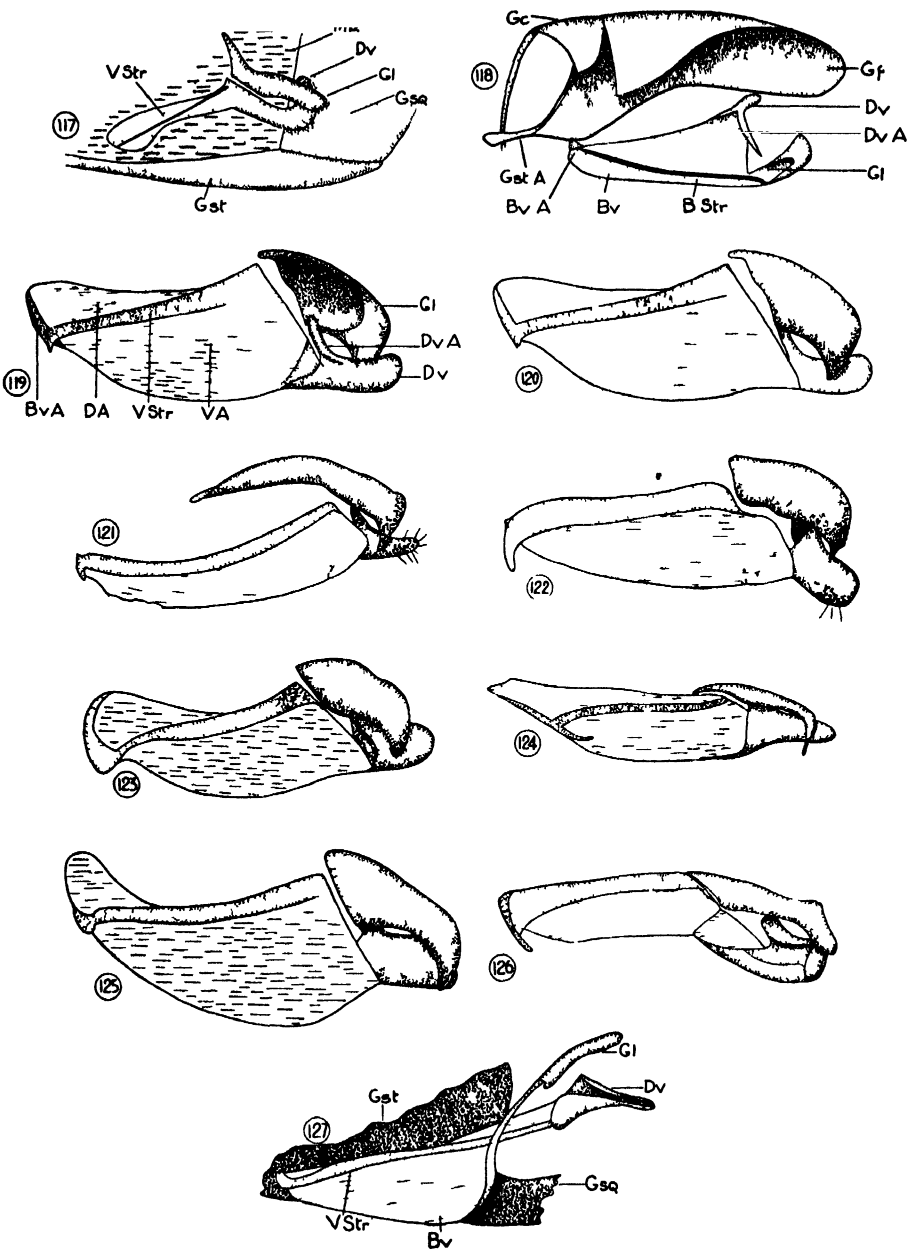
FIGS. 70–81. *The genitalia.* 70. *Megarhyssa lunator. Ventral view.* 71. *Megarhyssa lunator. Dorsal view.* 72. *Banchus falcatorius. As above.* 73. *Ophion obscurus. As above.* 74. *Agrypon flaveolatum. As above.* 75. *Exochilum circumflexum. As above.* 76. *Cremastus minor. As above.* 77. *Cremastus geminus. As above.* 78. *Cremastus flavo-orbitalis. As above.* 79. *Pristomerus vulnerator. As above.* 80. *Demophorus robustus. As above.* 81. *Orthopelma luteator. As above.*



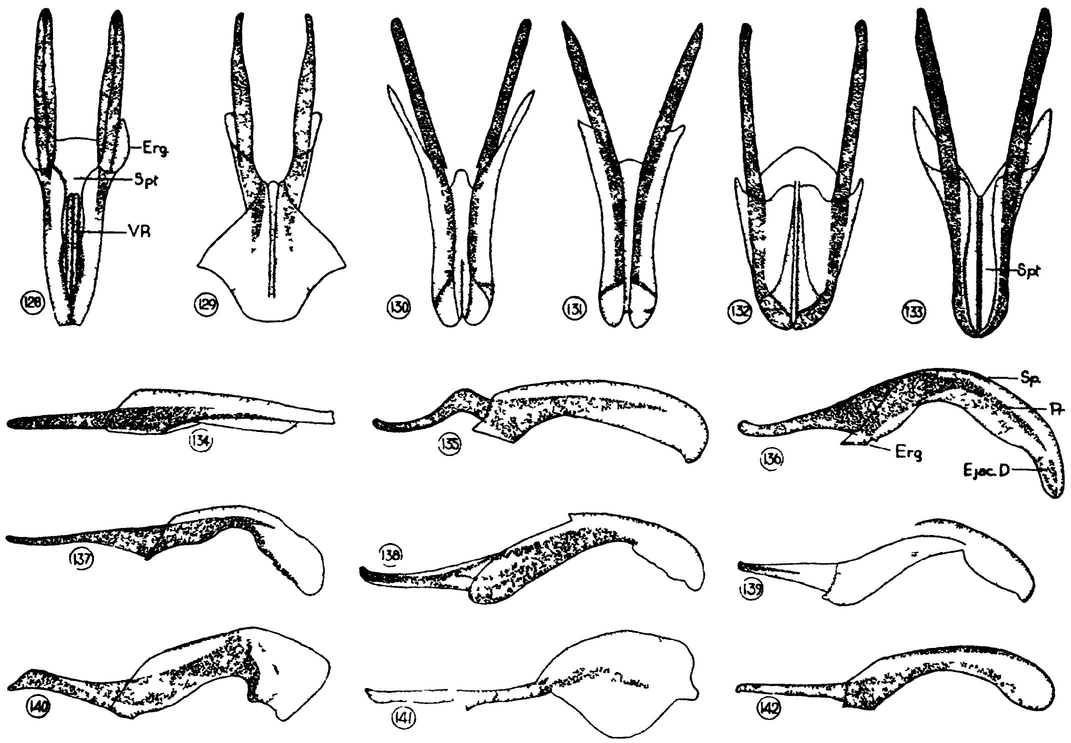
FIGS. 82–97. *The genitalia.* 82. *Mesochorus pectoralis. Dorsal view.* 83. *Hemiteles fulvipes. As above.* 84. *Hemiteles submarginatus. As above.* 85. *Enicospilus ramidulus. As above.* 86. *Apechthis ontario. As above.* 87. *Theronia fulvescens. As above.* 88. *Theronia melanocephala. As above.* 89. *Glypta rufiscutellaris. As above.* 90. *Glypta fumiferanae. As above.* 91. *Ephialtes tuberculatus. As above.* 92. *Ephialtes grapholithae. As above.* 93. *Exenterus canadensis. As above.* 94. *Exenterus claripennis. As above.* 95. *Exenterus lepidus. As above.* 96. *Exenterus marginatorius. As above.* 97. *Ichneumon longulus. Ventral view, showing the basal fusion of the volsella to the gonostipes.*



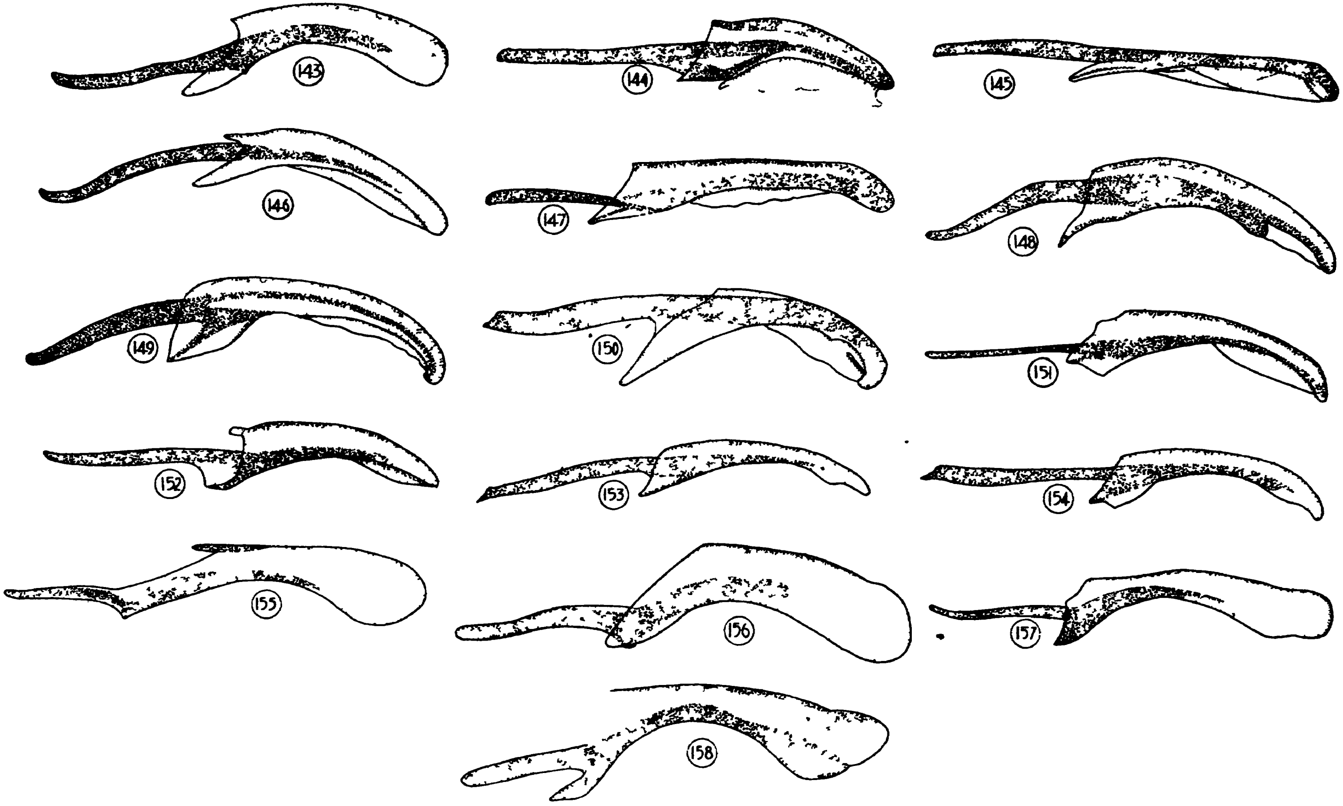
FIGS. 98–116. 98. *Hemiteles fulvipes. Lateral view of the genitalia.* 99. *Hemiteles submarginatus. As above.* 100. *Glypta rufiscutellaris. As above.* 101. *Glypta fumiferanae. As above.* 102. *Ephialtes tuberculatus. As above.* 103. *Ephialtes grapholithae. As above.* 104. *Cremastus interruptor. As above.* 105. *Cremastus geminus. As above.* 106. *Cremastus minor. As above.* 107. *Cremastus incompletus. As above.* 108. *Exenterus canadensis. As above.* 109. *Exenterus claripennis. As above.* 110. *Exenterus lepidus. As above.* 111. *Exenterus marginatorius. As above.* 112. *Megarhyssa lunator. As above.* 113. *Oryssus sayi (Oryssidae). As above, but the gonocardo absent.* 114. *Ichneumon longulus. Posterior view of gonocardo.* 115. *Ichneumon variegatus. As above.* 116. *Megarhyssa citraria. Dorsal view of gonocardo.*



FIGS 117–127 *The volsella* 117 *Tenthredella mesomelas (Tenthredinidae) Ventral view of the volsella in the horizontal plane and the adjacent sclerites (redrawn and shaded after Boulangé, (17)* 118 *Dolerus similis Internal view of the widely separated volsella and gonoforceps.* 119 *Neotypus americanus Lateral or internal view* 120. *Neotypus americanus. Mesal or external view* 121. *Ichneumon grotei. As above but showing portion of the basivolsella, torn from the gonostipes* 122 *Ichneumon animosus External view.* 123 *Hemiteles hemipterus. As above* 124. *Enicospilus ramidulus. As above* 125 *Cryptus annulatus. As above.* 126. *Cremastus minor. As above.* 127. *Cremastus incompletus. As above but showing the adjacent parts of the gonoforceps.*



FIGS. 128–142. *The aedeagus.* 128. *Megarhyssa lunator. Ventral view.* 129. *Agrypon flaveolatum As above.* 130. *Glypta fumiferanae. As above.* 131. *Glypta rufiscutellaris. As above.* 132. *Theronia fulvescens. As above.* 133. *Theronia melanocephala. As above.* 134. *Megarhyssa lunator. Lateral view.* 135. *Ichneumon perscrutator. As above.* 136. *Ichneumon grotei. As above.* 137. *Ichneumon variegatus. As above.* 138. *Phaeogenes gaspesensis. As above.* 139. *Phaeogenes hariolus. As above.* 140. *Exochilum circumflexum. As above.* 141 *Agrypon flaveolatum. As above.* 142. *Glypta fumiferanae. As above.*



FIGS. 143–158. *Lateral views of the aedeagus.* 143. *Glypta rufiscutellaris.* 144. *Theronia fulvescens.* 145. *Theronia melanocephala.* 146. *Ephialtes grapholithae.* 147. *Ephialtes tuberculatus.* 148. *Pimpla brevicornis* 149. *Pimpla coelebs.* 150. *Pimpla detrita.* 151. *Pimpla instigator.* 152 *Itoplectis conquisitor.* 153. *Itoplectis obesus.* 154. *Itoplectis pedalis.* 155. *Exenterus canadensis.* 156. *Exenterus claripennis.* 157. *Exenterus lepidus.* 158. *Exenterus marginatorius.*

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THE MALE GENITALIA IN THE HYMENOPTERA (INSECTA), ESPECIALLY THE FAMILY ICHNEUMONIDAE[1]

II. TAXONOMY OF THE ICHNEUMONID GENITALIA

BY OSWALD PECK

Introduction

During the last twenty-five years, there has been considerable disagreement upon the identity of the type species of some of the early ichneumonid genera and, therefore, upon their correct generic names (28, 31, 73, 103, 104, 134, 136). The merits of some of these proposed names are at present under the consideration of the International Commission upon Zoological Nomenclature (125); the names used in this study are therefore those consecrated by time and employed by Schmiedeknecht (114–117). This is of advantage also in that the majority of the species studied in this paper are European.

The classification of the Ichneumonidae is also still unsettled. The most recent complete classification is that of Schmiedeknecht (114). This has been brought further into line with recent thought by his unfinished series of fascicles, supplementing his Opuscula Ichneumonologica; in these he has adopted some of the concepts of Cushman and Rohwer (32), dividing the tribe Pimplini (*s.l.*) (116).

The generic limits of many ichneumonid genera are very indefinite at the present time and this condition is clearly shown in this study, where both holarctic and nearctic species are used. As an example, the nearctic species of the genus *Ichneumon* L. (*i.e.*, *Amblyteles* Cush. *et al.*) frequently cannot be placed with certainty within any of the genera or subgenera recognized by European workers (30, 81), although Cresson (25) attempted to do this. Cushman's concept of the genus *Ichneumon* has been retained. Too, the genus *Hemiteles* of Schmiedeknecht (114, 115) was considered by him as a single unit, for the sake of convenience. It is a very large group, susceptible to subdivision, as shown by Roman (102) and Cushman (30). Similarly the genus *Pimpla*, as recognized by Schmiedeknecht (114), is now divided into a number of subgenera or genera (30, 116).

When possible, the type genus of each tribe was selected for study. While, from the systematic viewpoint, the type genus should be studied whenever possible, yet, when material is scarce, the substitution of another genus may not be always detrimental and, in some cases, even advantageous. The type genus was arbitrarily created the nomenclatorial representative of a higher group, although possibly atypical. The type species is the systematic representative of a group but that is no criterion of its possession of the morphological attributes primitively common to the majority of that group. While in theory the characteristics of a group should be shown by a graduated series, in practice such thoroughness is often limited by time, material, and expense in publication.

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[1] *Concluded from November issue.*

These limitations have prevented any close study of specific differences, so that variations termed specific may prove to be only intra-specific. The practical restraints of material also preclude the thorough testing of any taxonomic inferences, therefore suggestions of this nature must be regarded as *purely tentative*, requiring the evaluation of the intensive systematist. This is needed also because the author claims no intimate knowledge of the various groups within the ichneumonids.

In order to aid the systematist to evaluate the data presented in this paper, the names of the ichneumonid species studied are listed below, the genera and species being grouped alphabetically in their respective tribes and subfamilies as recognized by Schmiedeknecht (114), subject to his revision of the Pimplinae (115). Cushman's division of the Pimplini (29) has also been indicated. Since the name *Ephialtes* L. is here used in the traditional sense for certain members of the Pimplini, all species of *Ephialtes* Cush. are referred to by his subgeneric name *Itoplectis*, here used with generic status merely to avoid a confusion in names.

Ichneumonidae

1. *ICHNEUMONINAE*
    - A. Joppini
        - *Trogus lutorius Fabr.*
    - B. Ichneumonini
        - *Ichneumon animosus* Cress.; *Ichneumon grotei* Cress.; *Ichneumon longulus* Cress.; *Ichneumon perscrutator* Wsm.; *Ichneumon variegatus* Cress.; *Neotypus americanus* Cush.
    - C. Phaeogenini
        - *Phaeogenes gaspesensis* Prov.; *Phaeogenes hariolus* Cress.
    - D. Alomyini
        - *Alomyia debellator* Fabr.
2. *CRYPTINAE*
    - A. Stilpnini
        - *Atractodes* sp.
    - B. Phygadeuonini
        - *Glyphicnemis crassipes* Prov.; *Microcryptus basizonius* Grav.; *Neostricklandia sericata* Cush.; *Stylocryptus subclavatus* Say.
    - C. Hemitelini
        - *Cecidonomus inimicus* Grav.; *Hemiteles (Astomaspis) fulvipes* Grav.; *Hemiteles (Astomaspis) submarginatus* Bridgm.; *Hemiteles (Hemiteles) hemipterus* Fabr.; *Hemiteles (Hemiteles) subzonatus* Grav.

D. Pezomachini
    No representative.
E. Cryptini (including Mesostenini)
    Acrornicus junceus Cress.; *Cryptus sexannulatus* Gr.

3. *PIMPLINAE*
A. Lissonotini
    Cylloceria sexlineata Say; *Lissonota varia* Cress.
B. Glyptini
    Glypta fumiferanae Vier.; *Glypta rufiscutellaris* Cress.
C. Lycorini
    No representative.
D. Phytodietini
    Phytodietus annulatus Prov.
E. Theroniini
    Theronia fulvescens Cress.; *Theronia melanocephala* Cress.
F. Polysphinctini
    Polysphincta sp. (*P. venator* De Gant?); *Zatypota percontatoria* Grav.
G. Labenini
    No representative.
H. Rhyssini
    Megarhyssa citraria Ol.; *Megarhyssa greenei* Vier.; *Megarhyssa lunator* Fabr.; *Rhyssa persuasoria* L.
I. Acoenitini
    Phaenolobus arator Rossi
J. Xoridini
    Deuteroxoides vittifrons Cress.
K. Odontomerini
    Odontomerus canadensis Prov.; *Odontomerus pinetorum* Thoms.
L. Pimplini
    (a. Pimplini Cush.)
    Ephialtes (Calliephialtes) grapholithae Cress.; *Ephialtes tuberculatus* auctt. Angl. nec Fourc.; *Exeristes roborator* Fabr.; *Pimpla brevicornis* Grav.; *Pimpla (Iseropus) coelebs* Walsh; *Pimpla detrita* Holmgr.; *Pimpla examinator* Fabr.; *Pimpla instigator* Fabr.
    (b. Ephialtini Cush. and Roh., nec auctt.)
    Apechthis ontario Say; *Itoplectis* (*Ephialtes* Cush.) *pedalis* Cress.; *Itoplectis (Itoplectis) conquisitor* Say; *Itoplectis (Itoplectis) obesus* Cush.

4. *TRYPHONINAE*
A. Mesoleptini
    Hadrodactylus typhae Geoffr.; *Lagarotus insolens* Grav.; *Lophyroplectus luteator* Thunb.; *Mesoleius multicolor* Grav.; *Mesoleius tenthredinidis* Morl.; *Perilissus (Spanotecnus) filicornis* Grav.

B. Tryphonini

Erromenus crassus Cress.; *Tryphon incestus* Holmgr.

C. Ctenischini

Exenterus canadensis Prov.; *Exenterus claripennis* Thoms.; *Exenterus lepidus* Holmgr.; *Exenterus marginatorius* Fabr.

D. Paniscini

No representative.

E. Bassini

Bassus tricinctus Grav.; *Homotropus pectoratorius* Grav.

F. Orthocentrini

Orthocentrus sp.

G. Exochini

Exochus sp.; *Triclistus curvator* Fabr.

H. Metopiini

Metopius sp.

5. OPHIONINAE

A. Ophionini

Enicospilus ramidulus Grav.; *Ophion obscurus* Fabr.

B. Nototrachini

Nototrachys foliator Fabr.

C. Ophionellini

Hymenopharsalia foutsi Cush.

D. Anomalonini

Agrypon flaveolatum Grav.; *Anomalon flavifrons* Grav.; *Exochilum circumflexum* L.

E. Campoplegini

Diocles obliteratus Cress.; *Inareolata punctoria* Rom.; *Omorgus borealis* Zett.; *Omorgus ensator* Grav.; *Omorgus mutabilis* Holmgr.

F. Cremastini

Cremastus (Cremastus) flavo-orbitalis Cam.; *Cremastus (Cremastus) geminus* Grav.; *Cremastus (Cremastus) interruptor* Grav.; *Cremastus (Cremastus) minor* Cush.; *Cremastus (Zaleptopygus) incompletus* Prov.; *Demophorus robustus* Brischke; *Pristomerus vulnerator* Panz.; *Pristomerus appalachianus* Vier.

G. Porizonini

Orthopelma luteator Grav.

H. Plectiscini

No representative.

I. Banchini

Banchus falcatorius Fabr.; *Exetastes fascipennis* Cress.; *Exetastes fornicator* Fabr.; *Exetastes matricus* Prov.

J. Mesochorini

Mesochorus pectoralis Ratz.; *Cidaphus occidentalis* Cush.

The Value of the Genitalia in the Order Hymenoptera

The male genitalia have been widely used for taxonomic purposes in the principal pterygote orders, including the Hymenoptera. Among the latter group these appendages have been invaluable in some aculeate families, although they have not been used extensively in the Chalastogastra and Ichneumonoidea.

The ignoring of the male genitalia in these lower groups does not seem to be due to a lack of suitable characters, for the diversity of form has been shown by both Crampton (22) and Boulangé (17). Crampton, however, drew no systematic conclusions and did not include any specific studies but, when his work is compared with that of Boulangé, it is evident that good specific characters occur in *Cimbex*, *Xiphidria*, *Tenthredo*, *Dolerus* and *Cephus*, a wide representation of the Chalastogastra. Boulangé (17) considered the aedeagus to be the best source of specific characteristics, and Benson (9) found the shape of the ninth sternum in the tenthredinid genus *Athalia* to be specific in value, while the gonosquamae and parapenes were used by Rohwer (99) to separate the tenthredinid genera, *Lagium* and *Zalagium*. Among the nearctic sawflies, the genitalia are characteristic in some genera of the Allantinae (107), in species groups of *Dolerus* (105) and in individual species in *Empria* (106).

The separation of the Oryssidae from the Chalastogastra is supported by the radical difference in the resting position of the genitalia, those of the former always being concealed (38, 98), while those of the latter are infrequently so.

Differences have been observed among the ichneumonids, more especially the elongate gonosquama of *Banchus*, *Hemiteles nanus* Grav., *Lathrolestes* Foerst. and *Parabates* Foerst. (82), as well as those of the Mesochorini (21, 82, 114). The genitalia of *Amblyteles* sp., *Cryptus* sp. *Ichneumon lineator* Fabr., *Pimpla roborator* Fabr., *Psilosage ephippium* Holmgr. (a tryphonine) and *Metopius dissectorius* Panz, are dissimilar (21), as are those of *Hemiteles hemipterus* Fabr., *Collyria calcitrator* Grav. and *Pimpla detrita* Holmgr. (109), while those of *Calliephialtes* sp. (26) and of *Mesostenus* sp. and *Tryphon* sp. (8) differ again from each other.

The workers upon the aculeate groups have used the male genitalia extensively, especially in the Apoidea (87), Colletidae (72, 91), Andrenidae (5, 48, 49, 92, 112); Megachilidae (67–69), Bombidae (59, 86, 94, 95), Pompilidae (89), Chrysididae (90), Vespidae (10, 58), Tiphiidae (3, 60), Mutillidae (64, 65, 88) and the Sphecoidea (77, 78, 83, 93). Most of these workers confined themselves to a study of the hypandrium and of the undissected genital parts and found that the hypandrium and the apices of the outer claspers, of the inner claspers and of the aedeagus were extremely valuable in the identification of species. The situation seems to be adequately summed up by Mickel (65), who states, in reference to the Mutillidae, that:—

"the genitalia of the male have proved of inestimable value in settling certain points, especially in groups of males that are superficially alike. It has been possible to determine that certain external characters were of specific value by using the genitalia as a criterion. On the other hand, certain groups of males quite different in superficial appearance possess genitalia that are practically identical, so that these structures have been of assistance not only in the distinguishing of species, but also in determining the relationships of species, which is exceedingly important from the viewpoint of phylogeny."

As a consequence of the extensive specific differentiation, the occurrence of generic and supra-generic characters seems to be rare. Hagens (48) suggested such for groups among the Apoidea but they do not appear to be well substantiated by his data. Parker considered the posterior spines of the hypandrium in some Bemicini as generic, while the shape of the spatha served to distinguish all but two of the twelve American genera (77, 78). The presence of the pygopods has been used by both Saunders (112) and Radoszkowski (93) as the first step in separating the Sphecoids, although in the Lasiini (Formicidae) it is of generic interest (37). A marked similarity of the genitalia was noted among the Pseninae (Sphecidae) and among the Tiphiidae, although the apical portions are subject to specific variation (61). This similarity is true also of many other groups; for example, the gonoforcipes of *Andrena* and *Halictus* (Apoidea), as illustrated by Atwood (5), seem to form two very distinct groups, although they are closely related. There thus appear to be reasonable grounds for suspecting that generic and possibly super-generic characters are present in the ichneumonid genitalia. Such an occurrence may be true in the Sphecoidea, for a key to the group was made by Radoszkowski (93), using chiefly the form of the inner clasper, the parapenial area and the gonosquama. However, few species were used in this key.

The existence of these higher characters is suggested also by the work of Boulangé (17), who stated that the basal portions of the chalastogastrous genital sclerites were of greater value in determining the larger groups than were the apices, owing to the restraint placed upon differentiation by the muscular attachments. Should this be so, it is not surprising that few characters of the higher denominations have been found, since most of the workers cited appear to have studied the genitalia as an undissected unit.

In contrast to these views, Richards (94, 96) holds that (i) almost any sclerites are liable to differentiation in the genus *Bombus* and (ii) in each of the main divisions of the aculeate groups, specialization takes place through fusion and, as this may occur within a family or even within a genus, it is not possible at present to use the male genitalia to any great extent in classification. While these views are not diametrically opposed to those of Radoszkowski, yet they do suggest that Radoszkowski's may need considerable modification and possibly even rejection.

Considering the evaluation of the genitalia in other hymenopterous groups, it seems probable that among the Ichneumonidae (i) there are numerous specific differences, occurring chiefly among the apical portions of the sclerites, (ii) generic and suprageneric characters are rare, to be found probably in the basal parts of the sclerites and (iii) if the differences involve fusion, then they may be regarded as abnormally variable. The work of Ceballos (21) and Salt (109) shows that Berthoumieu (12) was incorrect in believing that there are no significant differences in the ichneumonid genitalia, but the former workers make no suggestion as to the possible taxonomic value of these structures.

The purpose of the taxonomic portion of this paper is to estimate the amount of variation that occurs in the genitalia of the ichneumonid males. The differences may be of real practical value, the quoted findings of Mickel (65) upon these structures in the mutillids then being perhaps equally applicable to the ichneumonids. On the other hand, the ichneumonid genitalia may be merely concealed structures, their differences only separating groups or species that are already readily recognized. This practical evaluation of the genital structures, however, must be left to the intensive systematist, only the type of variation being shown in this paper.

The Genital and Postgenital Terga among the Ichneumonids

As shown above, the hymenopterous gonotergum slowly evolved from the form of the pregenital tergum, first as gonotergites and subsequently as either a syntergum or a pair of syntergites, the syntergites later degenerating. This series of modifications occurring gradually in the Hymenoptera suggests that these structures should possess characters of value in identifying large groups within the ichneumonids, although the conclusions of Richards (96) in regard to the fusion of sclerites in the aculeates suggest otherwise.

Within the Ichneumonidae, the gonotergum is apparently always divided and it may be fused to the tenth tergum; the latter may be split into a pair of lateral tergites, although there is no significant reduction in the size of the syntergites. Unfortunately, these changes are not linked to the larger groups, for the inherent tergal characters, if present, appear to be concealed or partially masked by other influences, these being probably either the depressed or compressed shape of the abdomen, or the variations in the proportions of the hypandrium and of the gonopods, or both.

When compared with the specific variation of the hypandrium and the genital appendages, the compression or depression of the abdomen may be considered as distinctly inherent and, if so, there should be a correlation between the gonotergal form and the extreme knife-edge type of compressed abdomen. Among the ten most suitable species of the latter type (taken without regard for taxonomic considerations), *Ophion obscurus*, *Enicospilus ramidulus* and *Anomalon flavifrons* (all closely related), as well as *Hymenopharsalia foutsi*, have synterga, while *Acrornicus junceus*, *Banchus falcatorius* and *Lophyroplectus luteator* have syntergites; *Cremastus* spp., *Agrypon flaveolatum* and *Exochilum circumflexum* have a syntergum that is partially separated medianly. Evidently, if abdominal shape is an inherent factor in the modification of the genital tergum, it is completely dominated by more superficial characters and can be ignored.

The specific characteristics of the ninth and tenth terga are found in (i) the general proportions, (ii) the degree of inter-tergal fusion and (iii) the degree of the division in the tenth tergum.

Proportional variations appear to be common, some being shown in the lateral views of species belonging to the genera *Glypta*, *Theronia*, *Exetastes* and *Exenterus* (Figs. 23–30).

The degree of fusion between the terga may vary specifically, as shown in the complete fusion in *Glypta fumiferanae*, although this is incomplete anteriorly in *G. rufiscutellaris* (Figs. 23, 24). The latter condition is found also in *Cecidonomus inimicus*, while the closely related *Hemiteles* species have the intertergal fusion completed.

The division of the tenth tergum is not always uniform within a genus. *Cremastus* has a series of species that includes synterga, almost completely separated syntergites and intermediate forms (Figs. 31–34). In *Itoplectis conquisitor* the syntergum is completely fused medianly but in *I. obesus* it is incompletely so, while the syntergites of *I. pedalis* have completely fused intersegmentally. Similarly a wide variation occurs in *Pimpla*, since *P. examinator* and *P. instigator* possess synterga, and *P. brevicornis* syntergites with incomplete intersegmental fusion, while *P. detrita* and *P. coelebs* have separate tergites in both terga (Fig. 21). *P. detrita*, however, has been placed in *Epiurus* by Roman, as cited by Salt (109).

Apart from the exceptions already noted, the terga in each genus are of one type only. It is possible to consider three of the exceptions as heterogeneous genera, for *Cremastus* and *Pristomerus* have been divided into subgenera (27) and Pimpla contains both internal and external parasitoids (129). *Glypta*, however, is now considered as a homogeneous group. While great tergal variation occurs within the Phygadeuonini, Hemitelini, Pimplini, Cremastini, Pristomerini and Mesoleptini, yet in other tribes the tergal structures appear to be fairly uniform. Among the 22 species of the Ichneumoninae and Cryptinae (including 14 genera), 19 possess complete syntergites; however, two others, *Cecidonomus inimicus* and *Glyphicnemis crassipes* have the intertergal fusion incomplete, while the ninth and tenth terga of *Stylocryptus subclavatus* are divided into separate tergites. The terga of the other three subfamilies are remarkably varied, although tribally they show a uniformity of structure, apart from the exceptions already mentioned. The data are insufficient to show distinctly that the terga may be inherently constant within a tribe but, if this is so, then such uniformity of structure is usually either partially or entirely masked by specific differences.

During the study of the gonotergum, variations in the antecosta were seen, the anterior process in some Pimplinae being especially conspicuous. Since the antecosta is an internal ridge, serving as a place of attachment for muscles, it is unlikely that this structure would be greatly modified by the minor changes in the genitalia. The antecosta may therefore be a guide to the higher relationships of the ichneumonids.

No deliberate comparisons were made between the pretergal and gonotergal antecostae, yet they appeared to be similar, as is to be expected.

The genital antecosta may be (i) *linear* in form, as in *Exenterus marginatorius* (Fig. 30) (ii) *ovate* near the ventral margin, as in *Glypta rufiscutellaris* (Fig. 24), or (iii) *appendicular*, a somewhat quadrate process projecting anteriorly from near the ventral margin of the antecosta, as in *Exeristes roborator* (Fig. 20) and *Itoplectis conquisitor* (Fig. 35).

The tergal antecosta is linear throughout the Ichneumoninae, Cryptinae and Ophioninae, exclusive of a small process in *Hemiteles submarginatus* and an oval thickening in both *Mesochorus pectoratorius* and *Cidaphus occidentalis* (Mesochorini); the two latter exceptions stress the remoteness of the Mesochorini from the other tribes of the Ophioninae. *Exetastes*, too, is an exception, for in *E. matricus* and *E. fascipennis* the antecosta is lineo-ovate, although in *E. fornicator* it is linear; however, the genus is aberrant, being placed midway between the Ophioninae and the Pimplinae by Schmiedeknecht (117) and in the Lissonotini by Cushman (30).

The great uniformity of the antecosta among the Ichneumoninae, Cryptinae and Ophioninae is in strong contrast to the variation that occurs among the Pimplinae and Tryphoninae and emphasizes the heterogeneity occurring in the latter two.

Among the Pimplinae this diversity is indicated also by the primitively linear antecosta being characteristic of the Lissonotini, Odontomerini, Acoenitini and *Pimpla coelebs*, groups that are widely separated from each other. This suggests that they may be the primitive members of four different natural groups, more especially since these represent respectively the four original tribes of the Pimplinae, *i.e.*, the Lissonotini, Xoridini, Acoenitini and Pimplini as recognized by Gravenhorst (47), Holmgren (54), Ashmead (4) and Schmiedeknecht (114).

The ovate antecosta occurs in Glyptini and Phytodietini, while the pendant form is found in the Pimplini (*s.s.*), except *Pimpla coelebs*. The intermediate ovato-appendicular type occurs throughout the Theroniini, Polysphinctini, Rhyssini and Xoridini. The ovate forms suggest that the Glyptini and Phytodietini are moderately primitive, particularly since (i) strong lissonotine affinities have been noted in the Glyptini (Cushman and Rohwer (32)) and (ii) the Phytodietini have been grouped with the Lissonotini by Foerster(40), Ashmead (4) and Schmiedeknecht (114), although lately given tribal rank by Cushman and Rohwer (32) and Schmiedeknecht (116). Cushman and Rohwer (32), however, regard the Lissonotini and Glyptini as forming a group that is not at all closely related to the Phytodietini or to the rest of the subfamily, although akin to the Banchine *Exetastes*.

Cushman and Rohwer (32) also separate the Odontomerini from the Xoridini. *Odontomerus* spp. possess linear antecostae, while this structure is ovato-appendicular in *Deuteroxoides vittifrons*. Thus the tribe Odontomerini is more primitive than the Xoridini.

However, while the shape of the antecosta may assist in determining the tribal relationships within the Pimplinae, yet more data must be obtained before this structure is accurately evaluated in this group. The possibility of the antecosta being tribal in value is enhanced by its uniformity in the Ichneumoninae, Cryptinae and Ophioninae, the antecosta in the Tryphoninae also showing marked agreement with the taxonomic groupings.

Among the Tryphoninae the pendant antecosta occurs in the members of the Bassini, Metopiini and the Orthocentrini, although it was not seen in the Exochini. The first two tribes form the Tryphonides schizodonti and T. aspidopi respectively, while the latter two form the Tryphonides prosopi, these being supertribes recognized from early times by Holmgren (53), Morley (74) and Schmiedeknecht (114). The occurrence of a linear antecosta in *Exochus* sp. and an ovate one in *Triclistus curvator* (Exochini) perhaps indicates (i) that the Exochini are related to the Orthocentrini, being the more primitive portion of the supertribe, and (ii) that the antecosta is of tribal rather than supertribal value.

The majority of the Tryphoninae, however, occur in the Tryphonides homalopi, in which the antecosta varies from linear to lineo-ovate, the ovate form occurring rarely. Specific differences may occur, for in the four species of *Exenterus* studied, *E. canadensis* and *E. marginatorius* have an ovate antecosta, while *E. claripennis* and *E. lepidus* have lineo-ovate ones; it is difficult to know what weight to place upon these variations. However, it should be noted that the small number of Tryphoninae examined, together with the large number of subtribes recognized in this group does not justify any conclusions being drawn.

The same is perhaps true of all of the subfamilies, yet the shape of the antecosta in some groups is remarkably constant and does suggest that these basal structures may prove, upon closer study, to possess super-generic characteristics that are very seldom subject to masking by generic or specific variations. This possibility is augmented by the deplorable state of the taxonomy in this group, for, in view of this, it would indeed be surprising if the correlation between the antecostal form and the taxonomic opinions was higher, particularly since one cannot expect to be able to group the major divisions of any taxonomic group by employing merely one structure.

The Ichneumonid Hypandrium

As in most of the Hymenoptera, the posterior and lateral margins of the ichneumonid hypandrium is specific in value, this being shown in *Ichneumon, Phaeogenes, Hemiteles, Exetastes, Glypta, Theronia, Omorgus, Cremastus, Pimpla,* and *Exenterus* (Figs. 39–67). Desclerotization in the median line sometimes occurs, as in *Exetastes* spp., *Cremastus* spp. and in *Tryphon incestus* (Fig. 68). However, caution is advisable in the use of the shape of the hypandrium since intra-specific variations occur in *Exeristes roborator;* moreover, the curvature and outline of the ninth sternum can be altered by differences in the preparation of mounts. In *Megarhyssa*, there appeared to be no differences between the species, although the form in the genus is distinctive (Fig. 69).

While the greater portion of the hypandrium is specific in value, yet the antecosta with its median spiculum appears to be as significant as the tergal antecosta in revealing higher group characteristics, for there is a high negative correlation between the maximum width of the gonotergal antecosta and the length of the spiculum—if the gonotergal antecosta is linear, the spiculum is long, and *vice versa*. This correlation is almost perfect in the Ichneumoninae, Cryptinae and Ophioninae, and is high in the other two subfamilies. Since the antecostae and spicula are only roughly classified, it is surprising that the correlation should be so high. In no case was a positive correlation found.

As a source of specific differences, the hypandrium appears to be the most promising of the sclerites in the genitalia and the genital segment, not only because it varies in form, but because many of its characteristics may be seen without dissection and because its large size usually permits a reasonably easy dissection to the novice.

The Ichneumonid Gonocardo

As noted above, the annular gonocardo is formed by the fusion of two lateral semicircular sclerites, each differentiated from the base of a gonopod. Among the ichneumonids these gonopodites are broadest ventro-laterally or laterally, their apices tapering towards the median line of the abdomen.

The ventral tips of these sclerites are almost invariably fused together as a narrow bridge. This area of juncture is always narrow, even when the gonocardo is unusually broad ventro-laterally, as in *Megarhyssa lunator* (Figs. 70, 71, 112) and *Banchus falcatorius* (Fig. 72). Medio-ventrally there is sometimes a small internal ridge or apophysis, presumably the remnant of a suture existing before the fusion was completed; these are noticeable in the more heavily sclerotized gonocardines.

The dorsal portion of these gonopodites varies greatly in shape. A complete annulus is typical of the Ichneumoninae and of the less specialized tribes among the Ophioninae, while in the other families the meso-dorsal apices are usually joined by membrane alone. Unfortunately these generalizations are not entirely true of all groups, for both *Neotypus americanus* (Ichneumoninae) and *Stylocryptus subclavatus* (Cryptinae) are exceptional. Furthermore, as these genera are typical of their respective subfamilies, it is likely that many exceptions will occur in groups intermediate to these larger groups.

An intergradation of these types also occurs in the Ophioninae. The gonocardo is completely annular in the less specialized tribes, including the Ophionini, Nototrachini, Ophionellini, Anomalonini, Campoplegini, Cremastini and Pristomerini. The species *Ophion obscurus*, *Agrypon flaveolatum* and *Exochilum circumflexum* (Figs. 73–75) may suggest a tendency for the primitive gonocardo to be medio-dorsally broad in the longitudinal direction, although this is not true of all, *Enicospilus ramidulus* (Fig. 85) having a fairly narrow dorsal bridge. The same region appears to be consistently very narrow in the intermediate tribes Cremastini and Pristomerini (Figs. 76–80) and to have degenerated into membrane among the highly specialized tribes, Banchini, Porizonini and Mesochorini (Figs. 72, 81, 82). It is of interest to note that the completely annulate gonocardo of *Hymenopharsalia* (Ophionellini) agrees with the position assigned to it by Cushman (29).

It should be noted that the Porizonini are represented only by *Orthopelma luteator*, the genus being aberrant and related to the Pimplinae (114), although placed in the Porizonini by both Schmiedeknecht (114) and Cushman (30). The remaining two specialized tribes, Mesochorini and Banchini, are also perhaps only placed provisionally in the Ophioninae. The Banchini may be related to the Lissonotini through the genus *Exetastes*, which is placed with the Banchini by Schmiedeknecht (114) and in the Lissonotini by Cushman (30). The remoteness of the genus has been emphasized by earlier taxonomists including Handlirsch (50), who created a subfamily for the genus; this attitude is supported by the occurrence of Braconid characteristics in the respiration of the banchine larvae (129). The Banchini have been regarded as remote, for Foerster (40) and Viereck (135) raised the group to family rank. The remaining group, the Mesochorini, is differentiated from almost all other Ichneumonidae by the extremely elongate outer claspers in the males (Fig. 82); the peculiarly flattened head and enlarged areolet are also highly characteristic. When the apparently consistent habit of hyperparasitism is also considered, it appears that the Mesochorini may merit elevation to the rank of supertribe.

Upon these grounds it is evident that the more specialized tribes of the Ophioninae possibly may not be true members of the subfamily and, if so, the Ophioninae are characterized by a completely annulated gonocardo. However there were not enough species available in these tribes for this to be determined.

While the gonocardo undoubtedly possesses characteristics common to large groups within the Ichneumonidae, yet this structure also shows definite specific differences. Viewed in cross-section, the ventral portion may be straight, as in *Ichneumon longulus* (Fig. 114) or incurved as in *I. variegatus* (Fig. 115). The gonocardo, when seen from below, frequently exhibits variation, the medio-ventral portion being either straight, curved anteriorly or curved posteriorly. The anterior curve is present in *Cremastus* spp., (Figs. 76–78) although the form of this portion of the gonocardo may vary specifically. Within *Ichneumon*, *Phaeogenes* and *Hemiteles (Astomaspis)* both the straight and the posteriorly curved forms occur, the former in *I. grotei* (barely curved), *P. hariolus* and *H. fulvipes* (Fig. 83) and the latter in the other *Ichneumon* spp. examined, in *P. gaspesensis* and in *H. submarginatus* (Fig. 84). In the examined species of *Omorgus* and *Exenterus* this area was invariably straight.

Among the Pimplinae also, the ventro-median portion of the gonocardo is of specific interest. *Megarhyssa citraria* is readily distinguished from the American species, *M. lunator* and *M. greenei*, by the antero-ventral notch in the former being but half the length and V-shaped without parallel sides (Figs. 70, 116). Within the genus *Pimpla*, too, this area possesses diagnostic characters. A prominent antero-median lobe distinguishes *P. brevicornis* from the other species that were examined. Some *Pimpla* species, including *P. examinator*, *P. instigator* and *P. detrita*, are exceptional among the ichneumonids, for the two lateral halves (*i.e.*, the basal gonopodites) have not fused ventrally; unfortunately, this has weakened the gonocardo, making it difficult for the unwarned to extract the genitalia without separating the two halves of the gonocardo. *Apechthis ontario* (Fig. 86) *Itoplectis conquisitor* and *I. obesus* are distinguished by the median portion being bent posteriorly as a broad "V" but this is not true of *I. pedalis.*

Yet, while the gonocardo may vary within a genus, the similarity of this structure in some genera, notably *Ichneumon, Megarhyssa, Glypta, Cremastus* and *Exenterus* suggest that these specific differences have not entirely concealed some characters of higher value.

The Ichneumonid Gonoforceps

The tribe Mesochorini is usually distinguished from other ichneumonids by its singular, extremely elongate gonosquamae, although this character is possessed also by *Ctenopelma sanguineum* Prov. (Tryphoninae) and by some or all of the Ophionellini. One might expect that the gonoforcipes could be of use in identifying some of the larger groups. This, however, seems highly improbable, for while individual groups sometimes possess a common facies, yet only rarely, as in *Megarhyssa* and the *Mesochorini*, does one find that such facies are peculiar to one group.

This infrequence is due, at least in part, to the fact that each part of the gonoforceps may be differentiated specifically. The form of the gonostipital arm may vary within a genus, as in *Cremastus, Hemiteles (Astomaspis), Theronia, Glypta, Ephialtes* and *Exenterus* (Figs. 76–78, 83, 84, 87–96), as well as in *Ichneumon* and *Phaeogenes.* The main portion of the gonoforceps may vary in shape within a genus but seldom as radically as in *Ephialtes, Theronia* and *Exenterus.* The apex of the outer claspers often varies greatly within genera such as *Hemiteles, Glypta, Ephialtes, Cremastus* and *Exenterus* (Figs. 98–111).

The Ichneumonid Volsella

The ichneumonid volsella is a very flexible structure, this being necessitated by its function of finding, clasping and stretching the membrane lying adjacent to the female gonopore. Owing to this versatility of form in the individual, there seems to be no ideal method of mounting these structures without subjecting some part to possible distortion.

This part has been illustrated in several planes by Cushman (26), Becker (8), Ceballos (21) and Salt (109) and their works collectively show that it does vary considerably. However, as they dealt with thirteen species, representing eleven genera and all five of the traditional subfamilies, no taxonomic conclusions can be made from their work.

The typical ichneumonid volsella is similar to that of *Neotypus americanus* (Figs. 119, 120), the names of the parts being indicated in these figures. The area dorsal to the volsellar strut is termed, when present, the *dorsal area* of the volsella (D.A.), its margin almost invariably being supported anteriorly by the basivolsellar apodeme (Bv. A.).

While the volsella usually is completely separated by membrane from the gonoforceps, eleven exceptions were found, these representing four tribes in the Tryphoninae and one each in the Ichneumoninae, Cryptinae and Ophioninae. This character, therefore, appears to be widely spread, although intermittent. This peculiarity is evidently specific, at least in some groups, for a sclerotic tongue connects the antero-ventral part of the basivolsella to the gonostipes in *Ichneumon longulus* (Fig. 97), *I. grotei* and *I. variegatus*, although the volsella is free in *I. animosus* and in *I. perscrutator*. In the genus *Cremastus* also, *C. incompletus* and *C. geminus* have the volsella joined broadly to the gonostipes, although the other species examined in this genus have the volsella entirely separated. This latter condition is true also of *Neostricklandia sericata*, *Lagarotus insolens*, *Homotropus pectoratorius*, *Bassus tricinctus*, *Triclistus curvator* and *Metopius* sp. The fusion in the last species occurs for more than half of the total length of the volsella.

Among the Ichneumoninae, excepting *Neotypus americanus*, the basivolsellar apodeme is large, curving ventrally (Figs. 121, 122), and this is true also of the species of *Atractodes* (Stilpnini) examined. Among the Phygadeuonini the apodeme is small but among the more specialized Cryptinae, such as *Hemiteles hemipterus* (Fig. 123), the apodeme is large and extended dorsally. To this *Hemiteles subzonatus* is an exception; for the apodeme is small and appears to be ventro-lateral.

The size of the dorsal area is closely correlated with the dorsal growth of the basivolsellar apodeme, for it is absent in the five *Ichneumon* species, scarcely present in *Alomyia debellator* and narrow in the other Ichneumoninae, as well as in *Atractodes*. Among the Phygadeuonini, the dorsal area is moderate in size, while it is well developed in the more specialized Cryptinae, such as *Cryptus sexannulatus* (Fig. 125). In *Glyphicnemis crassipes* (Phygadeuonini), however, the apodeme is small and does not support the large dorsal area. While this correlation is true also of *Neotypus americanus* and *Hemiteles subzonatus*, the former has a large dorsal area and the latter none, thus disagreeing with their present taxonomic position. This suggests that there is a closer correlation between the form of the apodeme and that of the dorsal area than between the shape of these structures and the taxonomic grouping within the Ichneumoninae and Cryptinae.

This conclusion also appears to be applicable to the other subfamilies, although, again, each subfamily has a strong tendency towards a particular type of apodeme with its corresponding form of the dorsal area.

Among the Tryphoninae, the basivolsellar apodeme is usually weakly developed, although it is strongly curved laterally in *Triclistus curvator* and ventrally in *Erromenus crassus*.

In the Ophioninae the apodeme is invariably ventral. The dorsal area is usually absent, except in *Orthopelma luteator*, *Exetastes* spp. and *Enicospilus ramidulus*. The first species has the apodeme developed dorsally, while the dorsal area is large. However, as the genus *Orthopelma* is aberrant (114), this case may be disregarded. In *Exetastes* spp. the dorsal area is present, although the apodeme is ventral and lateral; this genus is also aberrant (30, 114). In *Enicospilus ramidulus* (Fig. 124) the genitalia are unusually elongate so that this variation in volsellar form may be due to the need for reinforcement, for the apodeme is not of the normal dorsal type.

The form of the basivolsella divides the Pimplinae into two main groups, The Phytodietini being intermediate. The more specialized tribes are separated from the Lissonotini and Glyptini by the apodeme being well developed dorsally and by the great growth of the dorsal area. This agrees with the data upon the antecosta, which grouped together the Lissonotini and the Glyptini, as well as possibly the Phytodietini.

While the basivolsella is essentially a basal structure in which one may expect to find subfamily or tribal characters, yet it is also subject to generic or specific changes, not only in its fusion with the gonostipes, as already mentioned, but also in its proportions. The outline within some of the examined genera is similar, although specific differences may occur, as in *Ichneumon.* In this genus the narrowness of the basivolsella in *I. grotei* (Fig. 121) and *I. longulus* separates these species from *I. animosus* (Fig. 122), *I. variegatus* and *I. perscrutator.*

Among most of the Ichneumonidae, the apical portions of the volsella vary little from the type of *Neotypus americanus* (Figs. 119–120) although characters of specific value are to be found in the apex of the gonolacinia, for this may vary from being bluntly rounded, as in *Neotypus americanus* and *Glypta* spp. to being sharply pointed, as in *Ichneumon* spp. Within the genus *Ichneumon,* the length and shape of the gonolacinia varies, as shown in *I. grotei* (Fig. 121) and *I. animosus* (Fig. 122). A remarkable difference occurs in *Cremastus,* the gonolacinia being normal in both *C. minor* (Fig. 126) and *C. flavo-orbitalis,* although it is fused to the basivolsella in *C. incompletus* (Fig. 127) and *C. geminus.* This is of special interest as it emphasizes the fact that a character may appear to be extremely stable within the entire family although masked at times by a specific variation.

The Ichneumonid Aedeagus

The shape of the typical ichneumonid aedeagus is a weakly depressed cylinder, having the posterior apex curved somewhat ventrally and an elongate paramere extending anteriorly from each side. However, bizarre forms may occur within the family, as in *Agrypon flaveolatum* (Figs. 129, 141) and *Exochilum circumflexum* (Fig. 140).

The parameres are primitively joined to each other by membrane but the latter is often partially sclerotized, more particularly the dorsal or spathal area; this process is completed in some of the higher members of the Pimplinae, a medio-ventral fold (V.R.) being formed just in front of the apex (Figs. 70, 128, 134, 146–154). In most ichneumonids, however, this fold is membranous and allows the ventral portions of the valves to separate laterally. On this account the valves must be closed before comparisons are made in either the ventral or dorsal views.

The free anterior portion of the parameres is also subject to distortion, during treatment with potassium hydroxide, their shape therefore being of little taxonomic value. However, the anterior apex in *Hemiteles fulvipes* is dilated vertically, both upwards and downwards, in the form of a fishtail; in the other studied species of *Hemiteles* the paramere is rod-shaped, this being the almost invariable form in the Ichneumonidae. The length of the free portion may also vary specifically, as it is proportionately much less in *Ichneumon perscrutator* (Fig. 135) than in the other species studied in this genus (Figs. 136, 137).

These five species are readily divisible into three groups by their aedeagal shape. In both *I. grotei* (Fig. 137*) and *I. longulus* the aedeagus has small ergots and a dentate medio-ventral ridge, the ergots being long and the ridge without teeth in *I. variegatus* (Fig. 136), *I. animosus* and *I. perscrutator* (Fig. 135). The latter is readily distinguished from the others by the less falcate appearance and the shortness of the parameres.

All parts of the aedeagus seem to be differentiated specifically at times, as can be readily seen in *Hemiteles*, *Ichneumon*, *Phaeogenes*, *Glypta*, *Theronia*, *Ephialtes*, *Pimpla*, *Itoplectis* and *Exenterus* (Figs. 83–84, 130–133, 135–139, 142–158). The diverseness in form between species must conceal and offset to a very great extent any intrinsic character of higher value, although some of the species within a genus do appear to have a facies common to the aedeagus.

Summary

(1) The muscles of the male genitalia (and of the adjacent parts) in the ichneumonid, *Megarhyssa lunator* F., are shown to be homologous with those occurring in other hymenopterous groups, especially the Chalastogastra.

(2) The median genital organ is an aedeagus. The volsella and gonostipes are derived from the same primary part of the gonopod. The gonocardo may represent the fused coxopodites and the claspers the telopodite; the ontogenetic and myological data are not in agreement but the latter is probably much diminished in value by the genital muscles being aided by changes in blood pressure.

(3) The term *gonolacinia* should be used for the apical sclerite of the inner clasper, as a substitute for the word *sagitta*. The latter, used originally for structures now identified as parameres, is frequently misapplied.

(4) The ninth and tenth terga in the Chalastogastra are but little modified but those of the Aculeata are small because of their invagination. In the ichneumonids, the ninth is invariably divided into a pair of lateral tergites; these may be fused to the tenth tergum which may also be divided medianly.

(5) The ninth sternum in both the Chalastogastra and Ichneumonidae is large, possessing an antero-median appendage, the spiculum. In the higher Hymenoptera, the ninth sternum is small and sometimes fused to the eighth.

**The names I. grotei and I. variegatus are interchanged on the illustration on page 251.*

(6) Among the Ichneumonidae, almost every part of the genitalia (and of the adjacent parts) may be differentiated specifically. Variations that appear of value in identifying groups almost invariably have exceptions. While some of the latter may be due to misplacements of groups or species, the number of exceptions shows that specific variations mask characters of higher value to such an extent that the latter are unreliable.

(7) The form of the antecosta in the ninth tergum and the ninth spiculum may prove to have some value in the grouping of tribes. Unfortunately, these parts are not available for observation without dissection and treatment.

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STUDIES ON THE HETEROPHYID TREMATODE *APOPHALLUS VENUSTUS* (RANSOM, 1920) IN CANADA

PART III. FURTHER HOSTS[1]

By Thomas W. M. Cameron[2]

Abstract

Amia calva and *Ictalurus punctatus* are added to the list of carriers of the cysts of *Apophallus venustus* in the lower Ottawa Valley, and a probable human infection with the adult trematode is recorded.

Fish Hosts

During the summer of 1937, work was continued on the life history of *Apophallus venustus* with the result that two new fish have to be added to the list of carriers of the metacercaria given in Part II. of this series (1). These fish are the "fresh-water dogfish", "bowfin" or "lush" *(Amia calva)* and the "channel-cat" or "spotted catfish" *(Ictalurus punctatus).*

A number of specimens of both of those fish were caught by line in the Ottawa River, off Ste. Anne de Bellevue. As in the previous work, they were fed to cats which had been raised in the Institute and had not previously received any form of fish whatever.

The lush were not heavily infected but the catfish carried a considerable number of cysts. While it is not possible to give an exact comparison, they seem to be as suitable hosts as small-mouth black bass. This is of considerable interest as this species of catfish, like the bass, is an excellent food fish and a fair game fish. Various authorities rate it as being the most edible of fresh-water fish other than the Salmonidae.

Definitive Hosts

During the year, with the assistance of Dr. Angrove of the Ste. Anne de Bellevue Military Hospital, a considerable number of samples of human feces was submitted to the Institute for examination.

In one of these stools a number of eggs were found which agreed completely in shape and size with the eggs of *Apophallus venustus.* In spite of extensive search during the past three years no other trematodes of fish-eating mammals have yet been found in this neighborhood. Moreover, every species of this family of Trematodes, that has so far been tested in man, has found him a more or less suitable host.

While it is impossible to say conclusively that this patient was harboring some specimens of this parasite it is extremely probable that he was in fact doing so.

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